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Novel 4-thiazolidinone derivatives as potential antifungal and antibacterial drugs

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ABSTRACT

As part of ongoing studies in developing new antimicrobials, a class of structurally novel 4-thiazolidinone derivatives incorporating three known bioactive nuclei such as thiazole, thiazolidinone and adamantane was synthesized by the multi-step reaction protocol, already reported in the literature. NMR and Molecular Modeling techniques were employed for structure elucidation and *Z/E* potential isomerism configuration of the analogues. Evaluation of antibacterial and antifungal activity showed that almost all compounds exhibited better results than reference drugs thus they could be promising candidates for novel drugs.

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1. Introduction

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens with particular relevance for Gram positive bacteria.[1–5] On the other hand, a recent survey of novel small-molecule therapeutics revealed that the majority of them result from an analogue-based approach and that their market value represents two-thirds of all drug sales.[6]

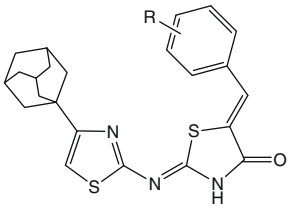
Because of this, and given our recent finding of a new class of antibacterial agents, the 2-thiazolylimino-5-benzylidene-4-thiazolidinones[7,8] we decided to extend our research to classes of analogues. It was found that 4-thiazolidinone ring system which is a core structure in various synthetic pharmaceuticals display a broad spectrum of biological activity, including antibacterial and antifungal properties.[9–14] Adamantane derivative have long been known for their antiviral activity against Influenza A[15–20] and HIV viruses.[21,22] Several adamantane derivatives were also associated with central nervous,[23–25] antimicrobial,[26–30] and anti-inflammatory activities.[31]

Our analogue-based design encompasses the synthesis of eleven new of 4-adamantyl-2-thiazolylimino-5-arylidene-4-thiazolidinone **5a**–**k** derivatives (Fig. 1), to be tested for their in vitro antimicrobial properties against Gram positive and Gram negative bacteria and fungi.

2. Results and discussion

2.1. Chemistry

As part of our ongoing studies in developing new antimicrobials[7,8] we report the synthesis of new class of structurally novel 4-thiazolidinone derivatives incorporating three known bioactive nuclei such as thiazole, thiazolidinone and adamantane.



5a: R = 4-Cl
5b: R = 3-Cl
5c: R = 2-Cl
5d: R = 4-NO_2
5e: R = 3-NO_2
5f: R = 2-NO_2
5g: R = 4-OH
5h: R = 4-OH, 3-OCH_3
5i: R = 4-OH, 3,5-OCH_3
5j: R = 4-OCH_3

Figure 1. Structure of the synthesized compounds.

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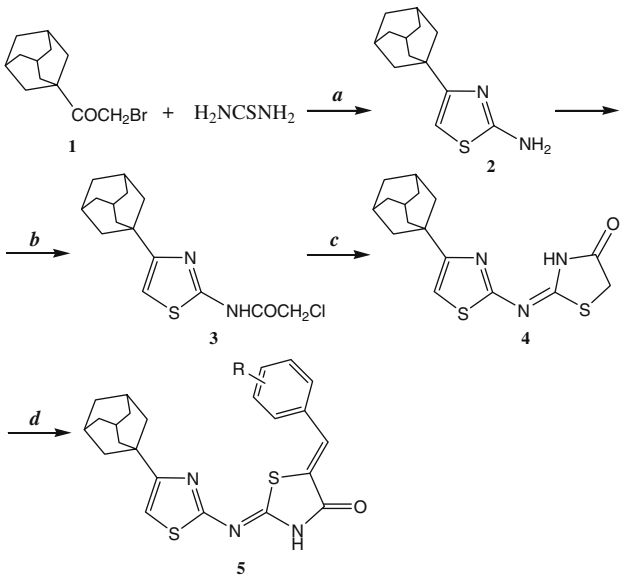
The compounds described in this paper were synthesized by the multi-step reaction protocol reported earlier by us[7] (Scheme 1). 2-Chloracetylchloride of 4-adamantyl-2-aminothiazole (**3**), synthesized using procedure reported earlier[32] from 4-adamantyl-2-aminothiazole (**2**), was undergone heterocyclization in the presence of ammonium isothiocyanate in refluxing ethanol[33,7] producing 2-(4-adamantylthiazol-2-ylimino)thiazolidin-4-one (**4**). The 4-adamantyl-2-thiazolylimino-5-arylidene-4-thiazolidinones **5a–j** were obtained by refluxing **4** with appropriate aldehydes in buffered glacial acetic acid (Scheme 1).

All the new compounds **5a–j** were characterized by mp, elemental analyses and spectroscopic data (^{1}H NMR, MS and IR). The spectral data and the elemental analysis of the new compounds reported in this study correlate with the proposed structures.

The mechanism of the cyclocondensation step, the amino–imino tautomeric equilibrium of the heteroarylthiazolidinones and of their adamantane derivatives **5a–5j**, the *E*/*Z* potential isomerism of the latter, were the same that was investigated through the analysis of IR and ^{1}H NMR spectral data for our compounds reported previously[7,8] and confirmed on the basis of the literature data.[34]

2.2. Antibacterial/antifungal activity

The results of antifungal activity of 4-[(adamantan-1-yl)1,3-(thiazol-2-ylimino)] thiazolidin-4-one **4** and its 5-arylidene derivatives compounds **5a–5j** against a panel of selected Gram positive, Gram negative bacteria and fungi are presented in Table 1 in comparison with those of the reference drugs ampicillin and streptomycin, bifonazole and ketoconazole, respectively.



5a: R = 4-Cl
5b: R = 3-Cl
5c: R = 2-Cl
5d: R = 4-NO_2
5e: R = 3-NO_2
5f: R = 2-NO_2
5g: R = 4-OH
5h: R = 4-OH, 3-OCH_3
5i: R = 4-OH, 3,5-OCH_3
5j: R = 4-OCH_3

Scheme 1. Synthesis of the compounds. Reagents and conditions: (a) isopropanol, stirring 0.5 h, rt: (b) $ClCOCH_2Cl$, *N*,*N*DMF, rt, 2 h; (c) NH_4SCN, EtOH, reflux, 1–2 h; (d) RC_6H_4CHO, MeCOOH, MeCOONa.

Compound **4** inhibited fungal growth at 0.60–2.38 $\times 10^{-2}$ μmol/ml while fungicidal activity was achieved at 2.38–3.35 $\times 10^{-2}$ μmol/ml. This compound showed the lowest antifungal activity, expressed as minimal inhibitory concentration (MIC) against *Penicillium funiculosum* (MIC 1.79 $\times 10^{-2}$ μmol/ml) *Aspergillus flavus* (MIC 1.79 $\times 10^{-2}$ μmol/ml) and *Aspergillus versicolor* (MIC 2.38 $\times 10^{-2}$ μmol/ml), moderate activity against *Trichoderma viride*, *Aspergillus fumigatus, Aspergillus niger* with MIC 1.19 $\times 10^{-2}$ μmol/ml, whereas it exhibited a strong effectiveness towards *Penicillium ochrochloron* and *Fulvia fulvum* (MIC 0.60 $\times 10^{-2}$ μmol/ml). In all cases activity of compound **4** was better than activity of two reference drugs, bifonazole and ketoconazole.

More significant inhibitory properties were detected for 5-arylidene derivatives **5a–j**.

All the compounds tested showed fungistatic activity at 0.52–2.38 $\times 10^{-2}$ μmol/ml and fungicidal at 1.05–3.35 $\times 10^{-2}$ μmol/ml against all the fungi tested. Derivatives **5a–5c** exhibited fungistatic effect at 0.55–2.19 $\times 10^{-2}$ μmol/ml and fungicidal activity was observed at 1.64–3.23 $\times 10^{-2}$ μmol/ml. In this group compound **5b** showed the best antifungal potential. Compounds **5d–5f** possessed almost the same activity, MIC at 0.54–2.15 $\times 10^{-2}$ μmol/ml, and MFC 1.08–2.15 $\times 10^{-2}$ μmol/ml. Derivatives **5g–5j** showed MIC at 0.52–1.73 $\times 10^{-2}$ μmol/ml and MFC at 1.05–2.31 $\times 10^{-2}$ μmol/ml, where compound **5i** exhibited the highest antifungal potential with MIC at 0.52–1.57 $\times 10^{-2}$ μmol/ml and MFC at 1.05–2.09. This compound showed the best antifungal effect among all the tested.

The majority of compounds showed the worst activity against *A. versicolor,* while *F. fulvum* is the most sensitive species. The most active compounds against this species (*F. fulvum*) among all tested are compounds **5i** (0.52 $\times 10^{-2}$ μmol/ml), followed by **5f** and **4**.

Taking into account that almost all compounds exhibited activity better than reference drugs, they could be promising candidates for antifungal drugs.

In addition compound **5a–j** were evaluated for antibacterial activity against a wide number of Gram positive bacteria, as well as Gram negative bacteria. The antibacterial activity of compounds tested by microdilution method, are presented in Table 2. The kind of the exerted antibacterial activity was investigated by determining the minimal bactericidal concentrations (MBCs). The experimental data (second values) presented in Table 2 show that 4-adamantyl-2-thiazolylimino-5-arylidene-4-thiazolidinones **5a–j** possess bacteriostatic properties, being MBCs almost twofold higher than the corresponding MICs.

All compounds showed strong antibacterial activity against all bacterial species. MIC for compounds **5a–5j** is at 1.05–4.76 $\times 10^{-2}$ μmol/ml and MBC at 1.57–7.17 $\times 10^{-2}$ μmol/ml. Compound **4** (initial thiazolidinone) exhibited the lowest antibacterial activity among all the other tested, with MIC 1.79–2.38 $\times 10^{-2}$ μmol/ml and MBC at 2.38–7.17 $\times 10^{-2}$ μmol/ml. Group of compounds **5a–5c** showed MIC at 1.10–4.38 $\times 10^{-2}$ μmol/ml and MBC 2.19–6.57 $\times 10^{-2}$ μmol/ml, where **5b** had the best activity. MIC of compounds **5d–5f** is at 1.08–4.30 $\times 10^{-2}$ μmol/ml and MBC at 2.15–6.45 $\times 10^{-2}$ μmol/ml. These compounds showed almost the same activity. Among derivatives **5g–5j** (MIC at 1.05–4.62 $\times 10^{-2}$ μmol/ml and MBC 1.57–6.93 $\times 10^{-2}$ μmol/ml) compound **5j** possessed the best antibacterial activity, MIC 1.05–4.18 $\times 10^{-2}$ μmol/ml and MBC at 2.09–6.27 $\times 10^{-2}$ μmol/ml. It can be seen that this compound in general showed the highest antibacterial activity. More significant inhibitory properties were detected for compound **5i** against *Micrococcus flavus* (ATCC 10240), *Staphylococcus aureus* as well as towards *Salmonella typhimurium* (MICs 1.05 $\times 10^{-2}$ μmol/ml).

The most sensitive bacterial species on compounds tested is Gram positive bacteria, especially, *Bacillus cereus*, while Gram negative bacteria *Pseudomonas mirabilis* is the most resistant species. It can be seen that all the compounds tested on antibacterial activity

Table 1
Antifungal activity of tested compounds and fungicides (MIC and MFC in μmol/ml × 10^{-2})

Fungi	**4** MIC MFC	**5a** MIC MFC	**5b** MIC MFC	**5c** MIC MFC	**5d** MIC MFC	**5e** MIC MFC	**5f** MIC MFC	**5g** MIC MFC	**5h** MIC MFC	**5i** MIC MFC	**5j** MIC MFC	Bif MIC MFC	Ket MIC MFC
P. funiculosum	1.79 2.38	1.10 2.19	2.19 2.19	2.19 3.23	2.15 2.15	1.08 2.15	1.08 2.15	1.72 2.29	1.64 2.19	1.05 2.09	1.16 2.31	64.0 80.0	38.0 95.0
P. ochrochloron	0.60 2.38	1.64 2.19	0.55 1.10	1.10 2.19	1.61 2.15	1.08 2.15	1.08 2.15	1.72 2.29	1.64 2.19	0.52 1.05	1.16 2.31	48.0 64.0	380.0 380.0
T. viride	1.19 2.38	1.10 1.64	1.10 2.19	1.10 2.19	1.08 2.15	0.54 1.08	1.08 2.15	1.15 2.29	1.10 2.19	1.05 2.09	1.16 2.31	64.0 80.0	475.0 570.0
A. fumigatus	1.19 2.38	1.10 2.19	1.10 2.19	1.64 2.19	1.61 2.15	1.61 2.15	1.08 2.15	1.15 2.29	1.64 2.19	1.57 2.09	1.73 2.31	48.0 64.0	38.0 95.0
A. niger	1.19 2.38	1.10 2.19	1.10 2.19	1.10 2.19	1.08 2.15	1.08 2.15	1.61 2.15	1.15 2.29	1.64 2.19	1.05 2.09	1.73 2.31	48.0 64.0	38.0 95.0
A. flavus	1.79 2.38	1.10 2.19	1.64 2.19	1.10 2.19	1.61 2.15	1.61 2.15	1.61 2.15	1.15 2.29	1.64 2.19	1.05 2.09	1.73 2.31	48.0 64.0	285.0 380.0
A. versicolor	2.38 3.35	1.64 2.19	1.10 2.19	1.64 2.19	1.61 2.15	1.61 2.15	1.08 1.61	1.72 2.29	1.64 2.19	1.57 2.09	1.73 2.31	32.0 64.0	38.0 95.0
F. fulvum	0.60 1.19	1.10 1.64	1.64 2.19	1.10 1.64	1.61 2.15	1.08 1.61	0.54 1.08	1.15 2.29	1.10 2.19	0.52 2.09	0.58 2.31	32.0 64.0	38.0 95.0

Table 2
Antibacterial activity of tested compounds and antibiotics (MIC and MBC in μmol/ml × 10^{-2})

Bacteria	**4** MIC MBC	**5a** MIC MBC	**5b** MIC MBC	**5c** MIC MBC	**5d** MIC MBC	**5e** MIC MBC	**5f** MIC MBC	**5g** MIC MBC	**5h** MIC MBC	**5i** MIC MBC	**5j** MIC MBC	Str MIC MBC	Amp MIC MBC
B. cereus	1.79 2.38	1.64 2.19	1.64 2.19	2.19 4.38	2.15 2.15	1.61 2.15	1.08 2.15	1.72 2.29	1.10 2.19	2.09 2.09	2.31 2.31	4.3 8.6	24.8 37.2
M. flavus	2.38 4.76	1.10 2.19	1.10 2.19	1.64 2.38	2.15 2.15	2.15 4.30	2.15 4.30	2.29 4.58	1.10 2.19	1.05 2.09	2.31 4.62	8.6 17.2	24.8 37.2
S. aureus	2.38 4.76	2.19 4.38	1.64 4.38	2.19 4.38	1.61 4.30	2.15 4.30	1.61 2.15	1.72 4.58	2.19 4.38	1.05 1.57	2.31 4.62	17.2 34.4	24.8 37.2
E. coli	2.38 4.76	2.19 4.38	2.19 4.38	2.19 4.38	2.15 4.30	2.15 2.15	2.15 4.30	2.29 4.58	1.10 1.64	2.09 4.18	1.16 2.31	17.2 34.4	37.2 49.2
P. aeruginosa	1.79 4.76	2.19 4.38	2.19 4.38	2.19 4.38	2.15 4.30	2.15 4.30	2.15 4.30	2.29 4.58	1.64 4.38	1.57 4.18	1.16 2.31	17.2 34.4	74.4 124.0
P. mirabilis	4.76 7.17	4.38 6.57	4.38 6.57	4.38 6.37	4.30 6.45	4.30 6.45	4.30 6.45	4.58 6.87	4.38 6.57	4.18 6.27	4.62 6.93	17.2 34.4	37.2 49.2
S. typhimuirium	2.38 4.76	2.19 4.38	2.19 4.38	2.19 4.38	2.15 2.15	1.08 2.15	2.15 4.30	1.15 1.72	2.19 4.38	1.05 1.57	1.16 2.31	17.2 34.4	24.8 49.2
L. monocyto	2.38 4.76	2.19 4.38	2.19 4.38	2.19 4.38	2.15 4.30	2.15 4.30	2.15 4.30	2.29 4.58	1.10 2.19	2.09 4.18	2.31 4.62	25.8 51.6	37.2 74.4

showed much better effect than commercial antibiotics, streptomycin and ampicillin (MIC 4.3–25.8 × 10^{-2} μmol/m and MBC 8.6–51.6 × 10^{-2} μmol/ml—for streptomycin, and MIC 24.8–74.4 × 10^{-2} μmol/ml and MBC 37.2–124.0 × 10^{-2} μmol/ml—for ampicillin). Thus all these compounds could be used as lead compounds for new antibacterial drugs.

Compounds **5i** in general showed the highest antibacterial as well as antifungal activity, while compound **4** exhibited the lowest antimicrobial potential.

It has been known that the introduction of arylidene moieties at different positions of the thiazolidinone ring enhanced the antimicrobial activity.[7,35,36] As regards the relationships between the structure and the detected antibacterial activity, the 5-arylidene derivatives **5a–j** showed a significant antibacterial efficacy, greater than that of parent 4-[adamantyl-2-(thiazol-2-ylimino)] thiazolidin-4-one **4** and appear to be dependent on the substitution at the benzene ring as well.

Thus, the chloro derivatives (**5a–c**) were found to be more effective against all tested microorganisms than arylidene derivatives carrying hydrophilic hydroxyl or methoxy group (**5g, 5j**) or nitro substituted compounds (**5d–f**).

It is interesting to point out that for the isomeric chloro substituted compounds (**5a–c**) the *meta* substituted derivative (**5b**) is mostly endowed with higher activity with respect to *ortho* and *para* (**5a** and **5c**) while for nitro substituted compounds **5d–f** the most active are *para* and *meta* (**5d** and **5e**) derivatives.

The introduction of methoxy group in position 3 and 3, 5 (**5h–i**) in the 4-hydroxy derivative (**5g**) in general lead to compounds with higher activity, whereas replacement of hydroxyl group with methoxy in the 4-position in most cases has the opposite effect. Only in case of Gram negative bacteria *P. aeruginosa* the replacement of hydroxy group in position 4 with methoxy (**5j**) enlarge the antimicrobial activity.

Almost the same behavior was observed for antifungal activity of tested compounds.

As a conclusion it can be noticed that fungi were in general more sensitive than bacterial species on these compounds.

3. Conclusion

The newly synthesized 4-adamantyl-2-thiazolylimino-5-arylidene-4-thiazolidinones **5a–j,** exhibit a remarkable inhibition of the growth of a wide spectrum of Gram positive bacteria, Gram negative bacteria and fungi. The most sensitive bacterial species on compounds tested is Gram positive bacteria, *B. cereus*, while Gram negative bacteria *Proteus mirabilis is the most resistant species.* As far as concern the fungi, the majority of compounds showed the worst activity against *A. versicolor*, while *F. flavum* is the most sensitive species.

It should be noticed that all compounds tested exhibited better activity than commercial antimicrobial agents used as reference drugs and few times higher activity than ketoconazole.

All the compounds **5a–j** showed excellent antibacterial activity indicating that the diverse substitutions were well tolerated on the benzylidene moiety for proper fit at the potential receptor site.

The outstanding properties of this new class of antibacterial substances deserve further investigation in order to clarify the mode of action at molecular level, responsible for the activity observed.

4. Experimental

4.1. Chemistry—general aspects

Melting points were taken in glass capillary tubes on a Haake Bucher apparatus and are uncorrected. IR spectra were recorded on a FT-IR Jasco spectrophotometer in solid phase KBr. All proton NMR spectra were determined with a Varian 300 MHz spectrometer using deuterated dimethylsulfoxide (DMSO-d_6) and are reported in δ (ppm) units. Thin layer chromatography (TLC) was performed in E. Merck precoated silica gel plates. Visualization was obtained by exposure to iodine vapors and/or under UV light (254 nm). The elemental analyses (C, H, N) of all compounds were performed by the Center of Instrumental Analysis of the University of Patras.

4.1.1. Synthesis of 4-adamantyl-2-aminothiazole (1)

To a solution of 1-adamantyl bromomethyl ketone, **1**, (257 mg, 1 mmol) in 5 ml of isopropanol, a suspension of thiourea (152 mg, 2 mmol) in 10 ml of isopropanol was added. The mixture was stirred for half an hour. After this time the resulting solution was poured into a solution of sodium carbonate, and the precipitate formed was filtered and dried to give, after recrystallization from ethylacetate, 220 mg (94%) of pure product, **2**. Mp 215–215.5 °C. IR (KBr): ν = 3050 cm^{-1} (N–H).

4.1.2. Synthesis of chloroacetylchloride of 4-adamantyl-2-aminothiazole

It was performed according to previous described method.[7]

4.1.3. Synthesis of 4-adamantyl-2-(thiazol-2-ylimino) thiazolidin-4-one (4)

A solution of 2-chloro-*N*-(4-adamnatyl thiazole) acetamide (5 mmol) and ammonium thiocyanate (10 mmol) in 20 mL of 96% ethanol was refluxed for 1 h and allowed to stand overnight. The precipitate was filtered washed with water and then recrystallised.

4.1.4. General procedure for synthesis of 4-adamantyl-2-thiazolylimino-5-arylidene-4-thiazolidinones 5a–j

A well-stirred solution of 0.8 g of 2-(4-adamantylthiazol-2-ylimino) thiazolidin-4-one (4 mmol) in 35 ML of glacial acetic acid was buffered with sodium acetate (8 mmol) and added with the appropriate arylaldehyde (6 mmol). The solution was refluxed for 4 h and then poured into ice-cold water. The precipitate was filtered and washed with water and the resulting crude product was purified by recrystallisation from dioxane.

4.1.4.1. (2*Z*,5*Z*)-2-{[4-(Adamantan-1-yl)-1,3-thiazol-2-yl]imino}-5-(4-chlorobenzylidene)-1,3-thiazolidin-4-one (5a). Yield: 76%. Mp 270–270.5 °C (dioxane). TLC: eluent = benzene–ethanol (9:1), R_f = 0.73. IR ν cm^{-1}: 3075 (NH), 1720 (C=O), 1592 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.71–2.07 (m, 15H, adam.), 6.95 (s, 1H, thiazolyl), 7.55 (d, 2H, ArH, J = 9 Hz), 7.66 (m, 2H, ArH and 1H, =CH), 12.63 (s, 1H, NH). Anal. Calcd for $C_{23}H_{22}N_3OS_2Cl$ (MW 455.5): C, 60.59; H, 4.82; N, 9.22. Found: C, 60.54; H, 4.80; N, 9.24.

4.1.4.2. (2*Z*,5*Z*)-2-{[4-(Adamantan-1-yl)-1,3-thiazol-2-yl]imino}-5-(3-chlorobenzylidene)-1,3-thiazolidin-4-one (5b). Yield: 86%. Mp 263–265 °C (dioxane). TLC: eluent = benzene–ethanol (9:1), R_f = 0.76. IR ν cm^{-1}: 3090 (NH), 1710 (C=O), 1592 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.75–2.05 (m, 15H, adam.), 6.96 (s, 1H, thiazolyl), 7.51 (d, 2H, ArH, J = 8.3 Hz), 7.65 (m, 2H, ArH and 1H, =CH), 12.68 (s, 1H, NH). Anal. Calcd for $C_{23}H_{22}N_3OS_2Cl$ (MW 455.5): C, 60.59; H, 4.82; N, 9.22. Found: C, 60.55; H, 4.83; N, 9.23.

4.1.4.3. (2*Z*,5*Z*)-2-{[4-(Adamantan-1-yl)- 1,3-thiazol-2-yl]imino}-5-(2-chlorobenzylidene)-1,3-thiazolidin-4-one (5c). Yield: 70%. Mp 214–215 °C (dioxane). TLC: eluent = benzene–ethanol (9:1), R_f = 0.64. IR ν cm^{-1}: 3100 (NH), 1720 (C=O), 1598 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.64–2.00 (m, 15H, adam.), 6.93 (s, 1H, thiazolyl), 7.42 (m, 1H, ArH), 7.49 (m, 1H, ArH), 7.63 (d, 1H, ArH, J = 8 Hz), 7.71 (d, 1H, ArH, J = 7.8 Hz), 7. 97 (s, 1H, =CH), 12.71 (s, 1H, NH). Anal. Calcd for $C_{23}H_{22}N_3OS_2Cl$ (MW 455.5): C, 60.59; H, 4.82; N, 9.22. Found: C, 60.65; H, 4.81; N, 9.21.

4.1.4.4. (2*Z*,5*Z*)-2-{[4-(Adamantan-1-yl)-1,3-thiazol-2-yl]imino}-5-(4-nitrobenzylidene)-1,3-thiazolidin-4-one (5d). Yield: 64%. Mp 285–287 °C (dioxane). TLC: eluent = benzene–ethanol (9:1), R_f = 0.74. IR ν cm^{-1}: 3080 (NH), 1715 (C=O), 1599 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.70–2.07 (m, 15H, adam.), 6.95 (s, 1H, thiazolyl), 7.75 (s, 1H, =CH), 7.88 (d, 2H, ArH, J = 8.6 Hz), 8.24 (d, 2H, ArH, J = 8.7 Hz), 12.77 (s, 1H, NH). MS: 465 (M+, 100%), 331 (72), 310 (4), 280 (9), 264 (5), 257 (55), 240 (20), 226 (3), 98 (7), 88 (9), 78 (11), 74 (4), 61 (6). Anal. Calcd for $C_{23}H_{22}N_4O_3S_2$ (MW 466): C, 59.22; H, 4.72; N, 12.01. Found: C, 59.29; H, 4.71; N, 12.03.

4.1.4.5. (2*Z*,5*Z*)-2-{[4-(Adamantan-1-yl)-1,3-thiazol-2-yl]imino}-5-(3-nitrobenzylidene)-1,3-thiazolidin-4-one (5e). Yield: 54%. Mp 264–265 °C (dioxane). TLC: eluent = benzene–ethanol (9:1), R_f = 0.85. IR ν cm^{-1}: 3085 (NH), 1700 (C=O), 1599 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.73–2.04 (m, 15H, adam.), 6.97 (s, 1H, thiazolyl), 7.78 (t, 1H, ArH, J = 8 Hz), 7.81 (s, 1H, =CH), 8.10 (d, 1H, ArH, J = 8.5 Hz), 8.27 (d, 1H, ArH, J = 8.5 Hz), 8.47 (s, 1H, ArH), 12.74 (s, 1H, NH). Anal. Calcd for $C_{23}H_{22}N_4O_3S_2$ (MW 466): C, 59.22; H, 4.72; N, 12.01. Found: C, 59.15; H, 4.71; N, 12.00.

4.1.4.6. 2-{[4-(Adamantan-1-yl)-1,3-thiazol-2-yl]imino}-5-(2-nitrobenzylidene)-1,3-thiazolidin-4-one (5f). Yield: 68%. Mp 208–210 °C (dioxane). TLC: eluent = benzene–ethanol (9:1), R_f = 0.80. IR ν cm^{-1}: 3087 (NH), 1718 (C=O), 1600 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.70–2.00 (m, 15H, adam.), 6.82, 6.92 (ss, 1H, thiazolyl), 7.88 (s, 1H, =CH), 7.71 (m, 1H, ArH), 7.82 (m, 1H, ArH), 7.84 (d, 1H, ArH, J = 7.6 Hz), 8.18 (d, 1H, ArH, J = 8 Hz), 11.95,12.71 (ss, 1H, NH). Anal. Calcd for $C_{23}H_{22}N_4O_3S_2$ (MW 466): C, 59.22; H, 4.72; N, 12.01. Found: C, 59.25; H, 4.73; N, 12.02.

4.1.4.7. (2*Z*,5*Z*)-2-{[4-(Adamantan-1-yl)-1,3-thiazol-2-yl]imino}-5-(4-hydroxybenzylidene)-1,3-thiazolidin-4-one (5g). Yield: 38%. Mp 250–251 °C (dioxane). TLC: eluent = benzene–ethanol (9:1), R_f = 0.72. IR ν cm^{-1}: 3500 (OH), 3085 (NH), 1700 (C=O), 1580 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.62–1.98 (m, 15H, adam.), 6.78–6.97 (m, 3H, ArH and thiazolyl), 7.46–7.50 (m, 3H, ArH and =CH), 10.24 (s, 1H, OH), 12.41 (s, 1H, NH). Anal. Calcd for $C_{23}H_{23}N_3O_2S_2$ (MW 437): C, 63.15; H, 5.26; N, 9.61. Found: C, 63.20; H, 5.28; N, 9.62.

4.1.4.8. (2*Z*,5*Z*)-2-{[4-(Adamantan-1-yl)-1,3-thiazol-2-yl]imino}-5-(4-hydroxy-3-methoxy-benzylidene)-1,3-thiazolidin-4-one (5h). Yield: 32%. Mp 251–252 °C (dioxane). TLC: eluent = benzene–ethanol (9:1), R_f = 0.72. IR ν cm^{-1}: 3550 (OH), 3085 (NH), 1699 (C=O), 1589 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.70–2.00 (m, 15H, adam.), 3.81 (s, 3H, OCH_3), 6.86 (d, 1H, ArH, J = 8.2 Hz), 6.91 (s, 1H, thiazolyl), 7.15 (d, 1H, ArH, J = 8.3 Hz), 7.20 (s, 1H, ArH), 7.59 (s, 1H, =CH), 9.92 (s, 1H, OH),12.46 (s, 1H, NH). Anal. Calcd for $C_{24}H_{25}N_3O_3S_2$ (MW 467): C, 61.67; H, 5.35; N, 8.99. Found: C, 61.62; H, 5.37; N, 9.00.

4.1.4.9. (2*Z*,5*Z*)-2-{[4-(Adamantan-1-yl)-1,3-thiazol-2-yl]imino}-5-(4-hydroxy-3,5-dimethoxy-benzylidene)-1,3-thiazolidin-4- (5i). Yield: 49%. Mp 263–263.5 °C (dioxane). TLC: eluent = benzene–ethanol (9:1), R_f = 0.67. IR ν cm^{-1}: 3450 (OH), 3085 (NH), 1700 (C=O), 1594 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.69–2.00 (m, 15H, adam.), 3.81 (s, 6H, OCH_3), 6.93 (s, 1H, thiazolyl), 6.95 (s, 2H, ArH), 7.62 (s, 1H, =CH), 9.29 (s, 1H, OH), 12.47 (s, 1H, NH). Anal. Calcd for $C_{25}H_{27}N_3O_4S_2$ (MW 497): C, 60.36; H, 5.43; N, 8.45. Found: C, 60.41; H, 5.41; N, 8.47.

4.1.4.10. (2*Z*,5*Z*)-2-{[4-(Adamantan-1-yl)-1,3-thiazol-2-yl]imino}-5-(4-methoxy-benzylidene)-1,3-thiazolidin-4-one (5j). Yield: 43%. Mp 260–260.5 °C (dioxane). TLC: eluent = benzene–ethanol (9:1), R_f = 0.61. IR ν cm^{-1}: 3100 (NH), 1710 (C=O), 1598 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.71–2.05 (m, 15H, adam.), 3.81 (s, 3H, OCH_3), 6.91 (s, 1H, thiazolyl), 7.01 (d, 2H, ArH, J = 8.7 Hz), 7.64 (m, 2H, ArH and 1H, =CH), 12.5 (s, 1H, NH). Anal. Calcd for $C_{24}H_{25}N_3O_2S_2$ (MW 451): C, 63.85; H, 5.54; N, 9.31. Found: C, 63.91; H, 5.56; N, 9.34.

4.2. Biological evaluation

4.2.1. Test for antifungal activity

For the antifungal bioassays, eight fungi were used: *A. flavus* (ATCC 9643), *A. fumigatus* (plant isolate), *A. niger* (ATCC 6275), *A. versicolor* (ATCC 11730), *F. fulvum* (TK 5318), *P. funiculosum* (ATCC 36839), *P. ochrochloron* (ATCC 9112) and *T. viride* (IAM 5061). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research 'Siniša Stanković', Belgrade, Serbia.

The micromycetes were maintained on malt agar and the cultures stored at 4 °C and sub-cultured once a month.[37] In order to investigate the antifungal activity of the extracts, a modified micro dilution technique was used.[38–40] The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 μl per well. The inocula were stored at 4 °C for further use. Dilutions of the inocula were cultured on solid malt agar to verify the absence of contamination and to check the validity of the inoculum.

Minimum inhibitory concentration (MIC) determinations were performed by a serial dilution technique using 96-well microtiter plates. The compounds investigated were dissolved in DMSO (1 mg/ml) and added in broth Malt medium with inoculum. The microplates were incubated for 72 h at 28 °C, respectively. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs.

The fungicidal concentrations (MFCs) were determined by serial sub-cultivation of a 2 μl into microtiter plates containing 100 μl of broth per well and further incubation 72 h at 28 °C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. DMSO was used as a negative control, commercial fungicides, bifonazole (Srbolek, Belgarde, Serbia) and ketoconazole (Zorkapharma, Šabac, Serbia), were used as positive controls (1–3000 μg/ml). All experiments were performed in duplicate and repeated three times.

4.2.2. Test for antibacterial activity

The following Gram negative bacteria were used: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *S. typhimurium* (ATCC 13311), *Proteus mirabilis* (human isolate) and the following Gram positive bacteria: *B. cereus* (clinical isolate), *M. flavus* (ATCC 10240), *Listeria monocytogenes* (NCTC 7973), and *S. aureus* (ATCC 6538). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research 'Siniša Stanković', Belgrade, Serbia.

The antibacterial assay was carried out by microdilution method[37–39] in order to determine the antibacterial activity of compounds tested against the human pathogenic bacteria.

The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^5 CFU/ml. The inocula were prepared daily and stored at +4 °C until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum. All experiments were performed in duplicate and repeated three times.

4.2.3. Microdilution test

The minimum inhibitory and bactericidal concentrations (MICs and MBCs) were determined using 96-well microtitre plates. The bacterial suspension was adjusted with sterile saline to a concentration of 1.0×10^5 cfu/ml. Compounds to be investigated were dissolved in broth LB medium (100 μl) with bacterial inoculum (1.0×10^4 cfu per well) to achieve the wanted concentrations (1 mg/ml). The microplates were incubated for 24 h at 48 °C. The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The MBCs were determined by serial sub-cultivation of 2 μl into microtitre plates containing 100 μl of broth per well and further incubation for 72 h. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by Microplate manager 4.0 (Bio-Rad Laboratories) and compared with a blank and the positive control. Streptomycin (Sigma P 7794) and Ampicillin (Panfarma, Belgarde, Serbia) were used as a positive control (1 mg/ml DMSO). All experiments were performed in duplicate and repeated three times.

4.3. NMR spectroscopy

Ultra precision NMR tubes Wilmad 535–5 mm were used for the NMR experiments. Compounds **5a–5j** were dissolved in DMSO-d_6 and a series of experiments were performed using Varian 600 MHz spectrometer at 300 K. All data are collected using pulse sequences and phase-cycling routines provided in the Varian libraries. The ^{1}H spectral width was set to 8500 Hz at 600 MHz. Typically the 2D NOESY spectrum was acquired with 4096 data points in t_2 dimension, 64 scans, 256 increments in t_1 dimension, 150 ms mixing time and a relaxation delay of 1 s. Data processing including apodization with cosine square bell function, Fourier

transformation and phasing, were performed using Varian VNMR software.

4.4. Molecular modeling

Computer calculations were performed using Quanta software of Molecular Simulations on a Silicon Graphics O^2. Molecular Mechanics calculations were carried out using the CHARMm force field. Initial 3D structures were first minimized with steepest descents and then with Newton-Raphson algorithms using an energy tolerance of 0.01 kcal/mol^{-1} A^{-1}, to reach a local minimum. The dielectric constant (e) was set to 45 during minimization simulating the DMSO environment. To generate conformers, a random search procedure (random sampling) was applied. This method randomly changes all defined torsion angles within a predefined angular window. The range of the torsion angle varies during the search procedure and generates new conformations. CHARMm energy minimization is performed for each randomly altered conformation. In order to explore the preferred torsion angles that correspond to the lowest energy conformers and energy barriers of the molecules under study, stochastic search procedures (systematic grid scan) were used. This method initiates a grid scan search that generates conformations by varying specified torsion angles over a grid of equally spaced values. Intervals of 5° were applied for the rotation of one bond and 10° for simultaneous rotation of two bonds. During these searches, the predetermined torsion angle remained constant while minimization using 1000 steps of conjugate gradient algorithm was applied to 'relax' the whole molecule.

4.5. Stereochemistry and conformational properties

Stereochemistry on the C=N imino and C=C exocyclic double bonds is determined based on NMR spectroscopy. Although synthetic procedures may provide both *E* and *Z* isomers of compounds 5a–**5j**, during recrystallization procedure a pure product of one isomer may occur. This becomes apparent from NMR spectral peaks and their integration values for almost all compounds (except compound **5f**). More specifically, using DMSO-d_6 as solvent, only one peak was observed for NH at ~12.5 ppm (apart from compound **5f** where two NH signals are apparent at 12.71 ppm and 11.95 ppm) resulting in the predominant existence of one isomer. More specifically, *Z* configuration of the exocyclic C=C bond, in the 5-benzylidene derivatives was attributed, since the methine proton resonated, as expected, at higher chemical shift values due to the deshielding effect of the adjacent C=O, than it would do in *E* configuration isomers, because of the lower deshielding effect of 1-S as already was mentioned in our previous paper,[7,8] as well as in the literature.[34,35]

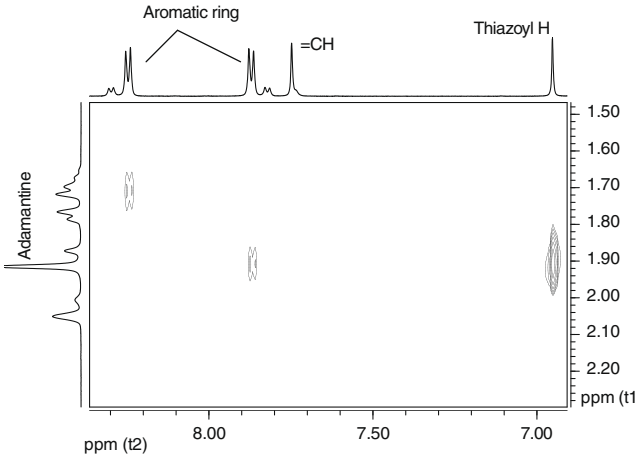


Figure 2. Expansion of NOESY spectrum of compound **5b** acquired at 600 MHz Varian. NOEs between adamantine and aromatic ring protons are indicative for the stereochemistry of the molecule.

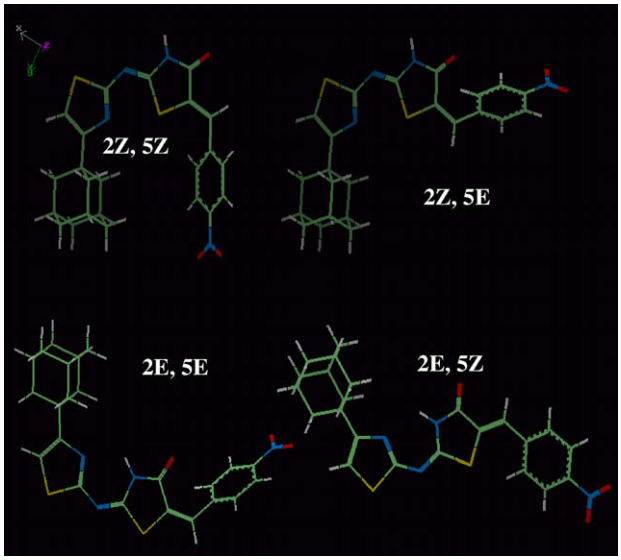


Figure 3. Representative low energy conformers of 2-{[4-(adamantan-1-yl)- 1,3-thiazol-2-yl]imino}-5-(4-nitrobenzylidene)-1,3-thiazolidin-4-one derived from Random Sampling conformational search, after clustering by torsions. Only 2*Z*,5*Z* configuration can satisfy the observed NOEs between adamantine and *para*-substituted aromatic ring protons.

Regarding the stereochemistry of the C=N imino exocyclic double bond, 2D NOESY NMR spectroscopy has shown NOE signals between adamantane and aromatic ring protons (Fig. 2). This experimental result suggests that these two moieties are close in space. Based on this finding, we proceeded with Molecular Modeling and conformational analysis studies in order to find geometries of the molecules to satisfy the observed NOEs. In conclusion, only 2*Z*,5*Z* isomers can satisfy this distance constrain which brings in proximity the adamantane and the aromatic ring (Fig. 3).

Assignment of the NH peak at ~12.5 ppm for the *Z* isomers on the C=N imino exocyclic double bond, results in the assignment of the NH peak at 11.95 ppm for the case of **5g**, to the *E* isomer.

The low energy resonance of the 1,3-thiazolidin-4-one NH is in accordance with previous results.[36] According to our opinion, intermolecular hydrogen bonds may occur.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.10.041.

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