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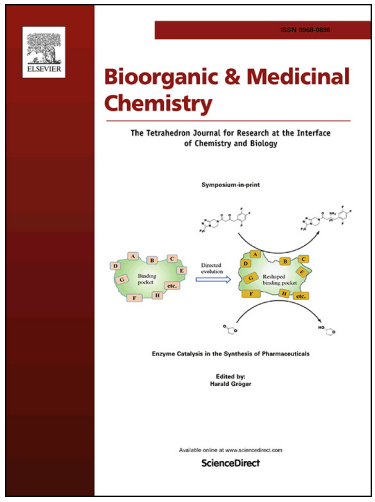

5-Adamantan thiadiazole-based thiazolidinones as antimicrobial agents. Design, synthesis, molecular docking and evaluation

Maria Fesatidou, Panagiotis Zagaliotis, Charalampos Camoutsis, Anthi Petrou, Phaedra Eleftheriou, Christophe Tratrat, Micheline Haroun, Athina Geronikaki, Ana Ciric, Marina Sokovic

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Graphical Abstract.

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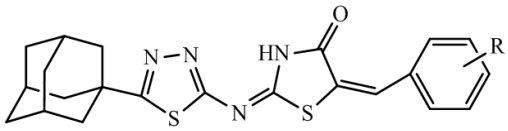
5-Adamantan thiadiazole- based thiazolidinones as antimicrobial agents. Design, synthesis, molecular docking and evaluation.

Maria Fesatidou[a] , Panagiotis Zagaliotis[a] , Charalampos Camoutsis[b] , Anthi Petrou[a] , Phaedra Eleftheriou[c], Christophe Tratrat[d] , Micheline Haroun[d] , Athina Geronikaki[a], Ana Ciric[e] , and Marina Sokovic[e]

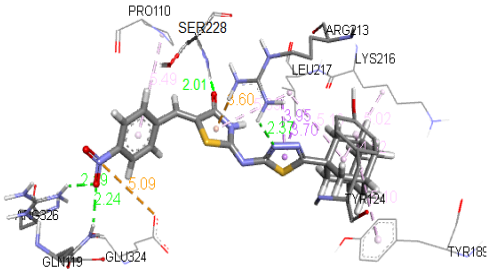
[a] *Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotle University of Thessaloniki, 54124, Greece*
[b] *School of Health Sciences, Department of Pharmacy, Laboratory of Pharmaceutical Chemistry, University of Patras, Greece .*
[c] *Department of Medical Laboratories, School of Health and Care Professions, Alexander Technological Educational Institute of Thessaloniki, 54700, Greece,*
[d] *Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Ahsa 31982, Saudi Arabia*
[e] *Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research, Siniša Stanković, University of Belgrade, Bulevar Despota Stefana 142, 11000, Belgrade, Serbia.*

Seventeen novel 2-{[5-(adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-arylidene-1,3-thiazolidin-4-ones were designed, synthesized and evaluated for antimicrobial activity. All compounds were potent antimicrobial agents. According to molecular docking studies, inhibition of MurB and CYP 51 may be involved in the mechanism of antibacterial and antifungal activities of most of the compounds.



Best compound anti-bacterial activity: MIC 1.65-3.30 $\cdot 10^{-2}$µM
anti-fungal activity: MIC 0.80-3-30 $\cdot 10^{-2}$µM

E.Coli MurB (PDB: 1Q85)



Compound 8: R= 4-NO_2 MIC : 2.20 10^{-2} µM

Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr

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5-Adamantan thiadiazole-based thiazolidinones as antimicrobial agents. Design, synthesis, molecular docking and evaluation.

Maria Fesatidou[a] , Panagiotis Zagaliotis[a] , Charalampos Camoutsis[b] , Anthi Petrou[a] , Phaedra Eleftheriou[c] Christophe Tratrat[d], Micheline Haroun[d] , Athina Geronikaki[a] , ,Ana Ciric[e] , and Marina Sokovic[e]

[a] *Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotle University of Thessaloniki, 54124, Greece*
[b] *School of Health Sciences, Department of Pharmacy, Laboratory of Pharmaceutical Chemistry, University of Patras, Greece .*
[c] *Department of Medical Laboratories, School of Health and Care Professions, Alexander Technological Educational Institute of Thessaloniki, 54700, Greece,*
[d] *Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Ahsa 31982, Saudi Arabia*
[e] *Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research, Siniša Stanković, University of Belgrade, Bulevar Despota Stefana 142, 11000, Belgrade, Serbia.*

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ABSTRACT

In continuation of our efforts to develop new compounds with antimicrobial properties we describe design, synthesis, molecular docking study and evaluation of antimicrobial activity of seventeen novel 2-{[5-(adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-arylidene-1,3-thiazolidin-4-ones. All compounds showed antibacterial activity against eight Gram positive and Gram negative bacterial species. Twelve out of seventeen compounds were more potent than streptomycin and all compounds exhibited higher potency than ampicillin. Compounds were also tested against three resistant bacterial strains: *MRSA, P. aeruginosa and E. coli.* The best antibacterial potential against ATCC and resistant strains was observed for compound **8** (2-{[5-(adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-(4-nitrobenzylidene)-1,3thiazolidin-4-one). The most sensitive bacterium appeared to be *S. typhimirium*, followed by *B. cereus* while *L. monocitogenes and M. flavus* were the most resistant. Compounds were also tested for their antifungal activity against eight fungal species. All compounds exhibited antifungal activity better than the reference drugs bifonazole and ketokonazole (3-115 times). It was found that compound **8** appeared again to be the most potent. Molecular docking studies on *E.coli* MurB, MurA as well as *C. albicans* CYP 51 and dihydrofolate reductase were used for the prediction of mechanism of antibacterial and antifungal activities confirming the experimental results.

1. Introduction

Bacterial infections are a growing problem worldwide causing morbidity and mortality mainly in developing countries. Some of the most deadly diseases such as salmonella, diarrhea and widespread epidemics as well as rheumatic fever and food poisoning result from bacterial infections.
The action of known antimicrobial drugs deals with inhibition of limited spectrum of cellular processes which are biosynthesis of proteins, RNA, DNA, cell wall and folic acid. Despite the continued success in discovery inhibitors against such targets [1,2] the frequency is low compared to the period of "golden era" of antibiotic drug discovery.[3]
Moreover, pathogenic bacteria have developed sophisticated intrinsic drug resistant mechanisms that are hard agitated in the microbial metagenome as a natural phenomenon and thus are difficult to fight.

Microorganism resistance to antimicrobials threatens the health of many people throughout the world, since both old and new infectious diseases remain a formidable public health threat. It should be mentioned that systemic fungal infections have progressively increased during the last decades. As a result, rates of morbidity and mortality, mainly due to the low effectiveness of available medications and the development of resistant strains, rose up. The problem becomes more serious in case of immune-compromised patients, who are more disposed to opportunistic fungal infections.
The treatment of such infections is quite difficult due to the spread of antifungal drug resistance generally in patients who are often subjected to antimytotic therapy and especially in immuno-compromised patients (cancer, transplants, AIDs).[4]

Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr

Thus, the increase in resistance of bacteria to antibiotics led the scientific community to focus on developing novel approaches to antimicrobial therapy. [5-11]

In this study we describe design, synthesis, molecular docking study and evaluation of antimicrobial activity of seventeen novel 2-{[5-(adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}5-aryliden-1,3-thiazolidin-4-ones.

Adamantane derivatives have been found to interfere with various enzymes and possess a variety of therapeutic activities, such as anti-inflammatory, [9-11] anti-viral, [13-15] anti-Parkinson, [16,17] antimicrobial, [18,19] anticancer, [14,20,21] CNS [22] as well as anti-HIV activity. [23,24]

Another interesting core is the thiazolidinone ring, also responsible for numerous pharmacological properties such as antimicrobial, [25-28] anti-inflammatory, [20, 29-31] anti-viral, [32-34] antioxidant, [35,36] anticancer, [37] antidiabetic [35 38] and antiarrythmic activities. [35] 4-thiazolidinones were recently found to exhibit antimicrobial activity, probably acting as inhibitors of MurB, thus inhibiting bacterial cell wall biosynthesis [38].

In addition, thiadiazole is heterocyclic system conferring antimicrobial, [40-42] antifungal [43-45] and a range of other activities, [46-50] possibly due to the presence of the toxophoric -N-C-S moiety. [51]

In general, thiadiazoles are a member of the big family of imidazole and triazole synthetic antifungal drugs designed to inhibit the enzyme cytochrome P450 14α-demethylase as well as the conversion of lanosterol to ergosterol, which is required in fungal cell membrane synthesis.

Several thiazolidinone derivatives were designed by introducing different arylidene substituents at the 5 position of the thiazolidinone moiety, which according to our previous observations [52] can be useful to encompass certain physico-chemical properties such as hydrophobic and steric.

2. Methods

2.1. Materials

All the chemicals used were of analytical grade and commercially available.

2.2. Synthesis of 1-(adamantyl-5-carboxy)thiosemicarbazide

To a stirred solution of thiosemicarbazide (0.91g, 0.01 mole) in dry pyridine (15mL) at -5°C a solution of 1-adamantanecarbonyl chloride (2.0g, 0.01 mole) in dry benzene (15 mL) was added. Stirring was continued for 0.5h at -5°C and then overnight at room temperature (25°C). The solvent was evaporated, water was added to the residue and the precipitate was filtered and crystallized from $CHCl_3$-CH_3OH to give 1.9g (75%) of the thiosemicarbazide. M.p.210-211 °C. Anal. Calc. for $C_{12}H_{19}N_3OS$ (MW 253.36): C: 56.88; H: 7.55; N: 16.59. Found (%): C: 56.85; H: 7.52; N: 16.54%.

2.3. Synthesis of 5-adamantyl-2-amino-1,3,4-thiadiazole

To well stirred and cooled (<5°C) concentrated sulfuric acid (10 mL), 1-(adamantyl-5-carboxy)thiosemicarbazide (1.1 g, 0.005 mole) was added in small portions. After the final addition the mixture was stirred for a further 15 min and was then allowed to reach ambient temperature (25°C), left stirring for a further 30 min and then poured cautiously onto crushed ice. The reaction mixture was alkalized to pH 8 with aqueous ammonia and the precipitated product was filtered off, washed with water and recrystallized from CH_3OH-CH_2Cl_2. Yield 87%. M.p. 200-201 °C. IR (cm^{-1}, KBr): 3050 (NH). ^{1}H-NMR: (δ ppm, DMSO, 500 MHz): 1.70-2.01 (m, 15H, adamant.), 7.01 (s, 2H, -NH_2).

2.4. Synthesis of N-[5-(1-adamantyl)-1,3,4-thiadiazol-2-yl)]-2-chloroacetamide

To a stirred solution of [(5-adamantan-1-yl)-2-amino-2-yl)]-1,3,4-thiadiazole (6.3 g, 0.03 mol) in anhydrous dimethyl formamide (64mL) , anhydrous sodium carbonate (3.2g, 0.08 mol) was added and then a solution of chloroacetyl chloride (9.0g, 0.080 mol) in anhydrous DMF (34.9 mL) was added dropwise. The reaction mixture was allowed to stir for 3 h at room temperature (25°C), then the resulting solution was poured onto ice water and the precipitate formed was filtered, washed with water, dried and crystallized from ethanol. Yield: 93%, m.p. 179-180 °C, Rf =0,73(petrol. ether-ethylacetate 1:1). IR (cm^{-1}, Nujol): 1712 (C=O), 3087 (NH). ^{1}H-NMR: (δ ppm, DMSO- d6, 300 MHz): 1.26 (s, 6H, adamantane) 1.52- 1.57 (d, 9H, adamantane), 3.91 (s, 2H, CH_2), 7.46 (s, 1H, NHCO). Anal. Calc. For $C_{14}H_{18}ClN_3OS$ (MW 311.82) C: 53.93, H: 5.77, N: 13.48. Found% C: 53.90, H:5.81,N:13.52%.

2.5. Synthesis of 2-[5-(adamantan-1-yl)-1,3,4-thiadiazol-2-yl]imino]-1,3-thiazolidin-4-one

To a solution of *N*-[5-(adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-2-chloroacetamide (0.005 mol) ammonium thiocyanate (0.01 mol) in ethanol 96%(20 mL) was added. The mixture was heated to reflux on a water bath for 1 h and left overnight. The resultant precipitate was filtered, washed with water and recrystallized from ethanol. Yield, 72%, m.p. 256-257°C. IR (cm^{-1}, Nujol): 3360 (νN-H), 1740 (C=O) (strong absorption), 1620 (aromat.). ^{1}H-NMR: (δ ppm, DMSO-d_6, 300 MHz): 1.75 (s, 6H, adamant), 1.99-2.06 (d, 9H, adamant), 4.07 (s, 2H, CH_2 thiazolid.), 12.20 (s, 1H, NHCO). Anal.calc. for $C_{15}H_{18}N_4OS_2$ (MW 334.45) C: 53.86; H: 5.42; N: 16.75%. Found: C: 53.83; H:.5.40; N:16.80%.

2.6. General procedure for synthesis of 2-{[5-(adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-arylidene-1,3-thiazolidin-4-ones[46]

To a well-stirred solution of 2-[5-(adamantan-1-yl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (0.001 mol, 0.33 g) in acetic acid (10 mL) buffered with sodium acetate (0.002 mol, 0.16g) the appropriate aromatic aldehyde (0.0015 mol) was added. The solution was heated to reflux for 4 h and then poured onto ice-cold water. The precipitate formed was filtered, washed with water and the resulting crude product purified by recrystallisation from dioxane.

2.6.1. 2-{[5-(Adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino} -5-(benzylidene)-1,3-thiazolidin-4-one (1)

Yield: 84%, m.p. 213-214°C (dioxane), R_f = 0.73 (toluene:-ethanol 8:2), IR: (cm^{-1}, Nujol): 1457 , 1653 (arom), 1717 (C=O), 3360 (NH). ^{1}H-NMR**:** (δ ppm, DMSO-d_6, 300 MHz): 8.89 (s, 1H, -NH) , 7.94 (t, 1H, C_4) , 7.86 – 7.89 (t, 2H, C_3, C_5) , 7.46 – 7.49 (d, 2H C_2, C_6) , 7.12 (s, 1H, -CH=) , 1.56 – 2.29 (m, 15H, adamantane). Anal.calc. for $C_{22}H_{22}N_4OS_2$ (MW 422.56) C: 62.53; H: 5.25; N:13.26%. Found: C: 62.49; H:5.29 ; N:13.20%

2.6.2. 2-{[5-(Adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-(4-hydroxy-benzylidene)-1,3-thiazolidin- 4-one (2)

Yield: 49%, m.p. 258-259°C (dioxane), R_f = 0.71 (toluene-EtOH 7:3), IR: (cm^{-1}, Nujol): 1570 (arom) , 1708 (C=O), 3089 (NH). ^{1}H-NMR**:** (δ ppm, DMSO-d_6, 300 MHz): 1.77 (s, 6H, adam.), 2.03-2.07 (d, 9H, adam), 6.95-6.98 (d, 2H, C_3, C_5), 7.51-7.53 (d, 2H, C_2,C_6), 7.67 (s, 1H, C-CH=), 10.22 (s, 1H, -OH), 12.60 (s, 1H, NHCO). ^{13}C-

Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr

NMR (500 MHz, DMSO-d_6): 28.44(3C), 31.78, 36.85(3C), 42.91(3C), 115.88(2C), 116.21, 126.12, 130.79(2C), 143.55, 158.32, 158.64, 159.21, 168.34, 173.28. Anal. Calcd. for $C_{22}H_{22}N_4O_2S_2$ (MW 438.56) C: 60.25; H: 5.06; N: 12.78%. Found%: C: 60.17; H: 5.01; N: 12.70%.

2.6.3. 2-{[5-(Adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}- 5-(4-methoxy-benzylidene)1,3-thiazolidin-4-one (3)

Yield: 62%, m.p. 222-223°C (dioxane), R_f = 0.74 (toluene-EtOH 7:3), IR: (cm^{-1}, Nujol): 1654 (arom) , 1717 (C=O), 3360 (NH). ^{1}H-NMR**:** (δ ppm, DMSO-d_6, 300 MHz): 8.79 (s, 1H, -NH) , 7.81-7.83 (d, 2H, C_2, C_6) , 7.15 – 7.17 (d, 2H, C3, C5) , 7.11 (s, 1H, -CH=), 3.78 (s, 3H, -OCH3), 1.56 – 2.27 (15H, adamantane). ^{13}C-NMR (500 MHz, DMSO-d_6): 28.12(3C), 31.75, 36.15(3C), 42.92(3C), 55.93, 114.59(2C), 116.67, 127.93(2C), 130.96(2C), 142.33, 158.81, 158.96, 159.17, 168.32, 174.21. Anal. Calcd. for $C_{23}H_{24}N_4O_2S_2$ (MW 452.59) C: 61.04; H: 5.34; N: 12.38%. Found%: C: 61.00; H: 5.31; N: 12.35%.

2.6.4. 2-{[5-(Adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}- 5-(3-methoxy-4-hydroxy-benzylidene)-1,3-thiazolidin-4-one (4)

Yield: 62%, m.p. 264-266°C (dioxane), R_f = 0.46 (petroleum ether: ethyl acetate 1:1), IR: (cm^{-1}, Nujol): 1600 (arom), 1711 (C=O), 3089 (NH). ^{1}H-NMR**:** (δ ppm, DMSO-d_6 300 MHz):): 1.77 (s, 6H, adam), 2.03-2.08 (d, 9H, adam.), 3.85 (s, 3H, -OCH_3), 7.00-7.03 (d, 1H, C_5), 7.15-7.17 (d, 1H, C_6), 7.29 (s, 1H, C_2), 7.72 (s, 1H, -CH=), 9.95 (s, 1H, -OH), 12.69 (s, 1H, NHCO). ^{13}C-NMR (500 MHz, DMSO-d_6): 28.44(3C), 31.78, 36.85(3C), 42.91(3C), 56.87, 111.85, 116.21, 116.93, 123.00, 128.51, 142.96, 148.11, 149.26, 158.32, 158.64, 168.41, 173.25.Anal. Calcd. for $C_{23}H_{24}N_4O_3S_2$ (MW 468.59) C: 58.95; H: 5.16; N: 11.96%.Found%: C: 58.86.; H: 5.20; N: 11.90%.

2.6.5. 2-{[5-(Adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-(4-methylbenzylidene)-1,3thiazolidin-4-one (5)

Yield: 58%, m.p. 217-218°C (dioxane), R_f = 0.74 (toluene-EtOH 8:2), IR: (cm^{-1}, Nujol): 1657 (arom) 1718 (C=O), 3360 (NH). ^{1}H-NMR**:** (δ ppm, DMSO-d_6 300 MHz): 9.34 (s, 1H, -NH) , 7.23 – 7.25 (d, 2H, C_2, C_5) , 7.11 – 7.13 (d, 3H, C_3, C_4, -CH=) , 2.39 (s, 3H,-CH_3), 1.56-2.29 (m, 15H, adamantane). ^{13}C-NMR (500 MHz, DMSO-d_6): 21.33, 28.36(3C), 31.66, 36.81(3C), 42.95(3C), 116.88, 128.43(2C), 128.66(2C), 132.03, 137.22, 142.83, 158.80, 159.16, 168.31, 173.74. Anal. Calcd. for $C_{23}H_{24}N_4OS_2$ (MW 436.59) C: 63.27; H: 5.54; N: 12.83%.Found%: C: 63.23; H: 5.50; N: 12.80%.

2.6.6. 2-{[5-(Adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-(2-nitrobenzylidene)-1,3-thiazolidin-4-one (6)

Yield: 71%, m.p. 239-240°C (dioxane), R_f = 0.77 (toluene-EtOH 8:2), IR: (cm^{-1}, Nujol): 1559 (νNO_2), 1654 (arom) 1717 (C=O), 3360 (NH). ^{1}H-NMR**:** (δ ppm, DMSO-d_6, 300 MHz): 8.84 (s, 1H, -NH) , 8.20 (d, 1H, C_5) , 7.92 (t, 1H, C_4) , 7.78 (d, 1H, C_2) , 7.71 (d, 1H, C_3) , 7.51 (s, 1H, -CH=) , 1.56 – 2.29 (m, 15H, adamantane). ^{13}C-NMR (500 MHz, $DMSO_3$-d_6): 28.43(3C), 31.72, 36.81(3C), 42.92(3C), 116.41, 123.66, 127.12(2C), 128.95, 134.81, 143.65, 147.22, 158.12, 159.11, 168.34, 173.68. Anal. Calcd. for $C_{22}H_{21}N_5O_3S_2$ (MW 467.56) C: 56.51; H: 4.53; N: 14.98%. Found (%):C: 56.48; H: 4.50; N: 14.94%.

2.6.7.2-{[5-(Adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-(3-nitrobenzylidene)-1,3-thiazolidin-4-one (7)

Yield: 69%, m.p. 257-258°C (dioxan), R_f = 0.76 (toluene-EtOH 8:2), IR: (cm^{-1}, Nujol): 1559 (NO_2),1653 (arom) 1717 (C=O), 3360 (NH). ^{1}H-NMR**:** (δ ppm, DMSO-d_6, 300 MHz): 9.29 (s, 1H, -NH) , 8.94 (s, 1H, C_2) , 8.30-8.32 (d, 2H, C_4, C_6) , 7.85 (t, 1H, C_5) , 7.22 (s, 1H, -CH=) , 1.01 – 2.49 (m, 15H, adamantane). ^{13}C-NMR (500 MHz,DMSO -d_6): 28.43(3C), 31.72, 36.81(3C), 42.91(3C), 116.01, 122.88, 123.02, 129.34, 134.23, 136.10, 143.66, 147.82, 158.13, 159.12, 168.34, 173.22. Anal. Calcd. for $C_{22}H_{21}N_5O_3S_2$ (MW 467.56) C: 56.51; H: 4.53; N: 14.98%. Found (%):C: 56.50; H: 4.55; N: 15.00%.

2.6.8.2-{[5-(Adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-(4-nitrobenzylidene)-1,3thiazolidin-4-one (8)

Yield: 72%, m.p. 306-307°C (dioxane), R_f = 0.76 (toluene-EtOH 8:2), IR: (cm^{-1}, Nujol): 1559 (NO_2),1654 (arom) 1717 (C=O), 3360 cm^{-1} (NH). ^{1}H-NMR**:** (δ ppm, DMSO-d_6 , 300 MHz): 8.68 (s, 1H, -NH), 8.10-8.12 (d, 2H, C_3, C_5), 7.75-7.77 (d, 2H, C_2, C_6) , 7.21 (s, 1H, -CH=) , 1.05 – 2.49 (m, 15H, adamantane). ^{13}C-NMR (500 MHz, $DMSO_3$-d_6): 28.43(3C), 31.72, 36.81(3C), 42.93(3C), 116.11, 123.11(2C), 129.37(2C), 140.92, 143.73, 147.11, 158.23, 159.16, 168.35, 173.35. Anal. Calcd. for $C_{22}H_{21}N_5O_3S_2$ (MW 467.56) C: 56.51; H: 4.53; N: 14.98%. Found (%):C: 56.53; H: 4.51; N: 14.97%.

2.6.9.2-{[5-(Adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-(4-dimethylaminobenzylidene)-1,3-thiazolidin- 4-one (9)

Yield: 75%, m.p. 292-293°C (dioxane), R_f = 0.76 (toluene-EtOH 8:2), IR: (cm^{-1}, Nujol): 1654 (arom), 1717 (C=O), 3360 (NH). ^{1}H-NMR**:** (δ ppm, DMSO-d_6 300 MHz): 9.34 (s, 1H, -NH) , 7.23 – 7.25 (d, 2H, C_2, C_5) , 7.11 – 7.13 (d, 3H, C_3, C_4, -CH=) , 2.39 (s, 6H, -CH_3), 1.56-2.29 (m, 15H, adamantane). Anal. Calcd. for $C_{24}H_{27}N_5OS_2$ (MW 465.63) C: 61.90; H: 5.84; N: 15.04%. Found (%):C: 61.89; H: 5.80; N: 15.00%.

2.6.10. 2-{[5-(Adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-(4-fluorobenzylidene)-1,3-thiazolidin-4-one (10)

Yield: 66%, m.p. 123-124°C (dioxane), R_f = 0.75 (toluene-EtOH 8:2), IR: (cm^{-1}, Nujol): 1653 (arom), 1717 (C=O), 3360 (NH). ^{1}H-NMR**:** (δ ppm, DMSO-d_6, 300 MHz): 7.86 – 7.89 (dd, 2H, C_3, C_5), 7.46 – 7.49 (t, 2H C_2, C_6), 7.12 (s, 1H, -CH=), 1.56 – 2.29 (m, 15H, adamantane). ^{13}C-NMR (500 MHz, DMSO-d_6): 28.11(3C), 31.76, 36.15(3C), 42.92(3C), 115.66(2C), 116.85, 130.96(2C), 131.57, 142.31, 158.81, 159.27, 160.88, 168.32, 174.13. Anal. Calcd. for $C_{22}H_{21}FN_4OS_2$ (MW 440.55) C: 59.98; H: 4.80; N: 12.72%. Found (%):C: 59.96; H: 4.83; N: 12.75%.

2.6.11. 2-{[5-(Adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-(2,6-difluorobenzylidene)-1,3-thiazolidin-4-one (11)

Yield: 51%, m.p. 239-240°C (dioxane, R_f = 0.78 (toluene-EtOH 8:2), IR: (cm^{-1}, Nujol): 1654 (arom), 1717 (C=O), 3360 (NH). ^{1}H-NMR**:** (δ ppm, DMSO-d_6 , 500 MHz): 9.14 (s, 1H, - NH) , 7.40 (m, 1H, C_4) , 7.18 (s, 1H, -CH=), 6.84 (t, 2H, C_3, C_5), 1.57 – 2.31 (m, 15H, adamantane)**.** ^{13}C-NMR (500 MHz, DMSO-d_6): 28.44(3C), 31.74, 36.85(3C), 42.91(3C), 111.37(2C), 112.98, 116.32, 129.77, 143.55, 158.02, 158.36(2C), 159.21, 168.24, 173.75. Anal. Calcd. for $C_{22}H_{20}F_2N_4OS_2$ (MW 458.55) C: 57.62; H: 4.39; N: 12.22%. Found (%):C: 57.61; H: 4.43; N: 12.25%.

2.6.12.2-{[5-(Adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-(4-bromobenzylidene)-1,3-thiazolidin-4-one (12)

Yield: 39%, m.p. 270-271°C (dioxane), R_f = 0.64 (petroleum ether: ethylacetate 1:1), IR: (cm^{-1}, Nujol): 1718 (C=O), 3067 (NH). ^{1}H-NMR**:** (δ ppm, DMSO-d_6, 300 MHz): 1.66-1.97 (m, 15H, adam.), 7.51-7.53 (d, 2H, C_3, C_5), 7.66 (s, 1H, -CH=), 7.69-7.72 (d, 2H, C_2, C_6), 11.97 (s, 1H, NHCO). ^{13}C-NMR (500 MHz, DMSO-d_6): 28.44(3C), 31.78, 36.85(3C), 42.91(3C), 116.11, 122.13, 128.56(2C), 131.79(2C), 133.22, 143.55, 158.32, 159.21, 168.34, 173.85. Anal. Calcd. for $C_{23}H_{21}BrN_4OS_2$ (MW 501.46) C: 52.69; H: 4.22; N: 11.17%.Found%: C: 52.75; H: 4.25; N: 11.14 %.

Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr

2.6.13.2-{[5-(Adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-(3-chlorobenzylidene)-1,3- thiazolidin-4-one (13)

Yield: 56%, m.p. 248-250°C (dioxane), R_f = 0.84 (petroleum ether: ethylacetate 1:1), IR: (cm^{-1}, Nujol): 1615 (arom), 1696 (C=O), 3092 (NH). ^{1}H-NMR**:** (δ ppm, DMSO-d_6 300 MHz): 1.72 (s, 6H, adam.), 1.98-2.02 (d, 9H, adam.), 7.58-7.72 (m, 5H, C_2, C_5, C_4, C_6, -CH=), 12.87 (s, 1H, NHCO). ^{13}C-NMR (500 MHz, DMSO-d_6): 28.43(3C), 31.75, 36.85(3C), 42.91(3C), 116.17, 126.38(2C), 128.11, 130.55, 134.21, 136.65, 142.13, 158.80, 159.17, 168.32, 174.05. Anal. Calcd. for $C_{22}H_{21}ClN_4OS_2$ (MW 457) C: 57.82; H: 4.63; N: 12.26%. Found%: C: 57.79; H: 4.65; N: 12.21%.

2.6.14. 2-{[5-(Adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-(4-chlorobenzylidene)-1,3- thiazolidin-4-one (14)

Yield: 59%, m.p. 263-264°C (dioxane), R_f = 0.67 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol): 1578 (arom), 1718 (C=O), 3110 (NH). ^{1}H-NMR**:** (δ ppm, $CDCl_3$, 300 MHz): 1.74 (s, 6H, adam.), 1.99-2.05 (d, 9H, adam.), 7.63-7.65 (m, 4H, C_2,C_3, C_5,C_6), 7.73 (s, 1H, -CH=), 12.82 (s, 1H, NHCO). ^{13}C-NMR (500 MHz, $CHCl_3$-d_6): 28.49(3C), 31.15, 36.85(3C), 42.91(3C), 116.03, 128.72(2C), 129.27 (2C), 133.01, 133.68, 142.95, 158.30, 159.15, 168.34, 173.39. Anal. Calcd. for $C_{22}H_{21}ClN_4OS_2$ (MW 457) C: 57.82; H: 4.63; N: 12.26%.Found%: C: 57.85; H: 4.58; N: 12.31 %.

2.6.15. 2-{[5-(Adamantan-1-yl)-1, 3, 4-thiadiazol-2-yl]-imino}-5-(2,3-dichlorobenzylidene)-1,3- thiazolidin-4-one (15)

Yield: 67%, m.p. 242-243°C (dioxane), R_f = 0.75 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol): 1654 (arom), 1717 (C=O), 3360 (NH). ^{1}H-NMR**:** (δ ppm, $CDCl_3$, 300 MHz): 7.62 (s, 1H, C_4), 7.15 (d, 2H, C_5, C_6), 7.12 (s, 1H, -CH=), 1.56 – 2.27 (m, 15H, adamantane). ^{13}C-NMR (500 MHz, $CHCl_3$-d_6): 28.77(3C), 32.41, 36.95(3C), 42.98(3C), 116.49, 125.34, 128.12(2c), 130.02, 134.52, 142.66, 143.19, 158.77, 159.34, 168.31, 174.18. Anal. Calcd. for $C_{22}H_{20}Cl_2N_4OS_2$ (MW 491.55) C: 53.75; H: 4.10; N: 11.40%. Found (%):C: 53.73; H: 4.12; N: 11.44%.

2.6.16. 2-{[5-(Adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-(2,4-dichlorobenzylidene)-1,3- thiazolidin-4-one (16)

Yield: 46%, m.p. 219-220°C (dioxane), R_f = 0.84 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol): 1654 (arom), 1717 (C=O), 3360 (NH). ^{1}H-NMR**:** (δ ppm, $CDCl_3$, 300 MHz): 8.94 (s, 1H, - NH), 7.62 (s, 1H, C_3), 7.15 (d, 2H, C_5, C_6), 7.12 (s, 1H, -CH=), 1.56 – 2.18 (m, 15H, adamantane). ^{13}C-NMR (500 MHz, $CHCl_3$-d_6): 28.15(3C), 32.12, 36.96(3C), 42.74(3C), 116.45, 125.31, 126.72 128.33(2c), 129.55, 130.11, 136.42, 143.08, 158.73, 159.24, 168.25, 173.97. Anal. Calcd. for $C_{22}H_{20}Cl_2N_4OS_2$ (MW 491.55) C: 53.75; H: 4.10; N: 11.40%. Found (%):C: 53.80; H: 4.00; N: 11.38%.

2.6.17. 2-{[5-(Adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-(2,6-dichlorobenzylidene)-1,3- thiazolidin-4-one (17)

Yield: 51%, m.p. 223-224°C (dioxane), R_f = 0.78 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol): 1654 (arom), 1717 (C=O), 3360 (NH). ^{1}H-NMR**:** (δ ppm, DMSO-d_6 300 MHz): 7.54 (d, 2H, C_3, C_5), 7.22 (s, 1H, -CH=) , 1.60 – 2.29 (m, 15H, adamantane). Anal. Calcd. for $C_{22}H_{20}Cl_2N_4OS_2$ (MW 491.55) C: 53.75; H: 4.10; N: 11.40%. Found (%):C: 53.75; H: 4.11; N: 11.41%.

3. Biological assays

3.1. Antibacterial activity

The following Gram-negative bacteria: *Escherichia coli* (ATCC 35210), *Enterobacter cloacae (clinical isolate), Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), as well as Gram-positive bacteria*: Listeria monocytogenes* (NCTC 7973), *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), and *Staphylococcus a*ureus (ATCC 6538) were used. The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research 'Siniša Stankovic, Belgrade, Serbia

The minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined by the microdilution method. Briefly, fresh overnight culture of bacteria was adjusted by the spectrophotometer to a concentration of 1×105 CFU/mL. Dilutions of inocula were cultured on solid medium to verify the absence of contamination and check the validity of the inoculum. Tested compounds were dissolved in 5% DMSO and added in broth Triptic Soy broth (TSB) medium (100 μL) with bacterial inoculum (1.0×104 CFU per well) to achieve the wanted concentrations (0.001-1.0 mg/ml) in dilution order. The microplates were incubated for 24 h at 370C. The MIC of the samples was detected following the addition of 40 μL of iodonitrotetrazolium chloride (INT) (0.2 mg/mL) and incubation at 37°C for 30 min. The lowest concentration that produced a significant inhibition of the growth of the bacteria in comparison with the positive control was identified as the MIC. 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. The minimum inhibitory concentrations (MICs) obtained from the susceptibility testing of various bacteria to tested extracts were determined also by a colorimetric microbial viability assay based on reduction of a INT color and compared with positive control for each bacterial strains. MBC was determined by serial sub-cultivation of 10 μL into microplates containing 100 μL of TSB. The lowest concentration that shows no growth after this sub-culturing was read as the MBC indicating 99.5% death of the original inoculum. Standard drugs, namely streptomycin and ampicillin were used as positive controls. Five % of DMSO was used as negative controls. All experiments were performed in duplicate and repeated three times.

The antibacterial assay was carried out by the microdilution method as previously reported.[53,54] All experiments were performed in duplicate and repeated three times.

3.2. Antifungal activity

For the antifungal bioassays, eight fungi were used: *Aspergillus niger* (ATCC 6275), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus fumigatus* (1022), *Aspergillus versicolor* (ATCC 11730), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112), *Trichoderma viride* (IAM 5061), and *Candida albicans* (human isolate). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research ''Siniša Stankovic,'' Belgrade, Serbia. All experiments were performed in duplicate and repeated three times.[55,56]

The micromycetes were maintained on malt agar and the cultures were stored at 4° C and sub-cultured once a month. The antifungal assay was carried out by modified microdilution technique. 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 1.0×105 in a final volume of 100 μL per well. The inocula were stored at 4° C for further use. Dilutions of the inoculum were cultured on solid malt agar to verify the absence of contamination and to check the validity of the

Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr

inoculum. MIC determinations were performed by a serial dilution technique using 96-well microtiter plates. The examined compounds were diluted in 5% of DMSO (0.001-1.0 mg/ml) and added in broth Malt medium (MA) with inoculum. The microplates were incubated at rotary shaker (160 rpm) for 72 h at 28° C. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs. The fungicidal concentrations (MFCs) were determined by serial subcultivation of 2 μL of tested fractions dissolved in medium and inoculated for 72 h, 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. The fungicides bifonazole and ketoconazole were used as positive controls (1-3500 μg/mL). Three independent experiments were performed in duplicate.

3.3. Statistical analysis

All the assays were carried out in triplicate and the results are expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 18.0 programs.

3.4. Docking studies

MurB, a common target of thiazolidinones was chosen for molecular docking studies in order to estimate the probable mechanism of antibacterial action. Among the available structures of MurB in the Protein Data Bank, the structure 2Q85 of *E.coli* MurB with its inhibitor, (5Z)-3-(4-chlorophenyl)-4-hydroxy-5-(naphthalen-1-ylmethylidene)furan-2-one, was chosen for docking analysis of the compounds. The MurB structures of *Pseudomonas aeruginosa* (4JAY and 4JB1), *Listeria monocytogenes* (3TX1) and *Staphylococcus aureus* (1HSK) are also available in the Data Bank and were also used for docking analysis of the most potent compounds. However, the structures 3TX1 and 1HSK correspond to enzymes crystallized without substrate or inhibitor and may not be the most appropriate for docking of potential inhibitors. Since conformational changes usually happen upon interaction of enzymes with substrates or inhibitors, structures derived from such complexes usually better present the domains needed for binding of the inhibitor.[57,58] The structure 4JAY of *Pseudomonas aeruginosa* MurB is derived from a complex of the enzyme with each co-enzyme FAD and NADPH which occupies the binding site of substrate or inhibitor and can be considered as appropriate for inhibitor screening. During enzyme preparation the inhibitor and the NADPH were abstracted from the structures of 2Q85 and 4JAY respectively. The co-enzyme, FAD, was maintained in all structures. The docking box was 50x50x50 Å in all cases. For the determination of the docking box center of the *E.coli* MurB (2Q85), the oxygen of -OH of the side chain of the catalytic amino acid Ser228 was used (x=7.534, y=-3.428 and z=11.525). The docking box was centered at x=47.65, y=-11.56 and z=14.08 for 4JAY, at x=-26.78, y=26.31 and z=-3.51 for 3TX1 and at x=180.81, y=148.25 and z =164.78 for 1HSK. The area of substrate/inhibitor binding and the co-enzyme FAD were always included in the docking box. The known MurB inhibitor Naphthyl Tetronic Acid was also docked for comparison reasons (Fig. 1S, supplementary files).
Docking of some compounds at the *E.coli* MuA (3KR6) was also performed. The docking box was set at $50 \times 50 \times 50$ Å and was centered at x=60.1366, y=46.2431 and z=148.6266 (center of the bound inhibitor fosfomycin) .

For the estimation of the probable mechanism of antifugal activity, all the synthesized compounds were docked in the active site of Dihydrofolate reductase 4HOF and CYP51 5V5Z of *C. albicans* (docking box centers: x=-0.895, y=0.131, z=32.109 and x=-47.731, y=-13.422, z=22.982, respectively). The human lanosterol 14a-demethylase CYP51 (3LD6), was also used for estimation of the selectivity of the compounds against the fungal enzyme. The docking box was also set at 50×50×50 Å and centered at x=39.272, y=8.184 and z=3.545. All selected structures were in complex with inhibitors. Docking of these inhibitors to their enzyme structures was performed for verification of the method (docking results at the supplementary files, Fig. 2S and 3S). The reference compound ketoconazole was also docked in the active site of 5V5Z structure.

Molecular Docking Studies of all compounds in *E.coli* MurB 2Q85, MuA (3KR6) and dihydrofolate reductase (4HOF) and CYP51 (5V5Z) of *C. albicans* were performed using the software Autodock 4.2. For the simulation, default values of quaternation, translation and torsion steps were applied. The Lamarckian Genetic Algorithm with default parameters was applied for minimization. The number of docking runs was 100. The graphical depictions of all ligand-protein complexes were consummated by Discovery studio visualizer version 4.0(BIOVIA, San Diego, CA, USA) and LigandScout 4.1.1. For the preparation of ligand structures, 2D structure was sketched in Chem Draw 12.0 and converted to 3D structure, mol2 format, for each ligand. Hydrogens were added to the structures and used for docking. All procedures were performed according to previous papers.[59,60] The online service of Molecular Docking Server, also based on AutoDock, was used for MurB enzymes of *Pseudomonas aeruginosa* (4JAY), *Listeria monocytogenes* (3TX1) and *Staphylococcus aureus* (1HSK) as previously described in our paper.[61]

4. Results and discussion

4.1. Prediction of Toxicity

As prediction of toxicity of a compound is a very important step in the design of new drug candidates, the *in silico* toxicity study can be used since it is a more rapid and less expensive process than *in vivo* toxicity testing in animals and *in vitro* testing in cell lines. Besides, it can help to significantly reduce the number of animals used in the experimental assays. There are several online programs using *in silico* models to access toxicity, that predict average lethal dose, carcinogenicity, mutagenicity etc.

Two computer programs, ToxPredict (OPEN TOX) and PROTOX, were used in this work .[62-64]

The ToxPredict program predicts the probability of carcinogenicity of the compounds in various organisms, as well as the probability of mutagenesis using an *in silico* model corresponding to the Ames test. The results are presented in Table 1S (Supplementary material). It should be mentioned that the accuracy of prediction increases as the confidence values rises. In particular, reliable estimates are considered to be more than 0.025.

The PROTOX program [58], predicts the average lethal dose (LD_{50}) in rodents. According to this program all chemical compounds can be classified into six GHS (Globally Harmonized System of Classification and Labeling of Chemicals) categories, [65] depending on the toxicity of the compounds and the LD_{50} values (Table 2S).

Category I: $LD_{50} \leq 5$ mg/kg

Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr

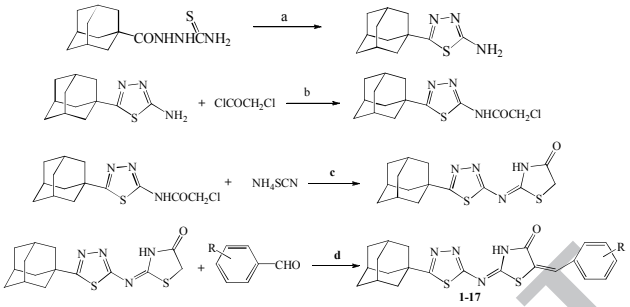
Category II: $5 < LD_{50} \leq 50$ mg/kg
Category III: $50 < LD_{50} \leq 300$ mg/kg
Category IV: $300 < LD_{50} \leq 2000$ mg/kg
Category V: $2000 < LD_{50} \leq 5000$ mg/kg
Category VI: $LD_{50} > 5000$ mg/kg

All compounds tested were in category IV.

4.2. Chemistry

The general method employed to prepare the final compounds is shown in scheme **1**. 5-Adamantyl-2-(1,3,4-thiadiazole)imino-5-arylidene-4-thiazolidinones were synthesized starting from 5-adamantyl-1,3,4-thiadiazole, prepared from the reaction of 1-adamantyl-5-carbonylchloride with thiosemicarbazide and subsequent cyclization of the intermediate 1-(1-adamantyl-5-carboxy)thiosemicarbazide in cold sulfuric acid (Scheme 1). 5-Adamantyl-1,3,4-thiadiazolyl-2-chloroacetamide, obtained by reacting of 5-adamantyl-1,3,4-thiadiazolyl-2-amine with chloroacetyl chloride, underwent heterocyclization in the presence of ammonium thiocyanate in refluxing ethanol leading to 2-{[5-(adamantan-1-yl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one. The 2-{[(5-adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-arylidene-1,3-thiazolidin-4-ones were obtained by heating to reflux the previous thiazolidinone with the appropriate aldehydes in buffered glacial acetic acid. Overall the reactions proceeded smoothly in good yields. Yields, physical properties and molecular formulas are given in the experimental part. Structures of all compounds were confirmed spectroscopically (IR, ^{1}H-NMR, ^{13}C-NMR, MS) and by elemental analyses.

The mechanism of cyclization with formation of thiazolidinone was mentioned in our previous paper.[46] The IR spectra were in agreement with the proposed structures, showing a sharp band in the region 1680–1720 cm^{-1} (NHC=O).The substitution position in the cyclocondensation step and the tautomeric structure of 2-imino-5-arylidene-4-thiazolidinones (**1-17**) were determined through the analysis of IR and ^{1}H NMR spectroscopic data. Thiazolidinones show, in their ^{1}H NMR spectra, a NH proton at 12.00-12.87 ppm, in accordance with a lactam proton and not with an imine proton (expected around 9.70 ppm).[66-68]



Com.	R	Com.	R
1	H	**10**	4-F
2	4-OH	**11**	2,6-di-F
3	4-OMe	**12**	4-Br
4	4-OH, 3-OMe	**13**	3-Cl
5	4-Me	**14**	4-Cl
6	2-NO_2	**15**	2,3-di-Cl
7	3-NO_2	**16**	2,4-di-Cl
8	4-NO_2	**17**	2,6-di-Cl
9	$N(CH_3)_2$		

Scheme 1. Synthesis of 2-{[5-(adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-arylidene-1,3-thiazolidin-4-ones (**a**: conc.H_2SO_4, <5°C(15 min), r.t.30 min; **b**: DMF, 3h, r.t.; **c**:NH_4SCN, EtOH reflux 1h, r.t. overnight; **d**: gl.CH_3COOH, CH_3COONa, refl.4h)

5. Biological evaluation

5.1. Antimicrobial activity

The antimicrobial activity of the synthesized compounds was evaluated using the microdilution method for determining the minimal inhibitory and minimal bactericidal/fungicidal concentrations.

Results of antibacterial activity of adamantane thiadiazole – based thiazolidinones. (**1**)-(**17**) are shown in Table 1. The antibacterial potential can be presented as follows: **8 > 6 > 7 > 11 > 1 > 2 > 3 > 17 > 5 > 4 > 9 > 16 > 12 > 15 > 13 > 10 > 14.**

The best antibacterial activity is achieved for compound (**8**), with MIC at 1.65-3.30 × 10^{-2} mmol/mL and MBC at 3.30-6.60× 10^{-2} microM. Compound (**14**) showed the lowest antibacterial efficacy with MIC and MBC at 3.30-32.8 × 10^{-2} microM and 6.60-82.20 × 10^{-2} microM, respectively. It should be mentioned that bacteria in general showed different sensitivities to compounds tested.

Thus, the most sensitive bacterium appeared to be *S. typhimirium*, followed by *B. cereus* while *L. monocitogenes and M. flavus* were the most resistant. Compounds (**1**), (**2**), (**6**), (**7**), (**8**), (**12**), (**14**) exhibited good activity against *S. typhimirium* with MIC at 1.40-3.40 × 10^{-2} microM and MBC at 3.30-6.60 x 10^{-2} microM, while some of them (**2,6,7,8,14)** with MIC and MBC at 2.14-3.40 and 4.28-6.60 × 10^{-2} microM respectively, were also potent against *P. aeruginosa*. Good activity was observed for compounds (**8**) and (**11**) against *En. cloacae*, as well as for compound (**8**) against *E. coli*. On the other hand (**6**-**8**) and (**12**) appeared to be active against the Gram positive bacterium *B. cereus*, while (**7**) and (**8**) were also potent against the most resistant bacterium *L. monocytogenes* as well as against *M. flavus* and *S. aureus,* (**7**) being more active than ampicillin and in some cases even than streptomycin. Compound (**7**) appeared to be about 29 times more active than ampicillin and 20 times than streptomycin against *L. monocytogenes.*

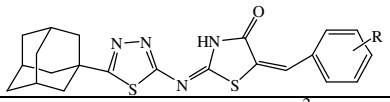
In particular, for the Gram-positive bacteria the range of MIC and MBC was 1.65-29.50 × 10^{-2}microM and 2.14-82.2 × 10^{-2} microM respectively, whereas for Gram-negative bacteria the MIC and MBC ranged from 1.40-29.50 × 10^{-2} microM, and 1.80 to 59.0 × 10^{-2} microM respectively. It seems that the tested compounds are more potent against Gram negative bacteria than against Gram positive.

It can be noticed that compounds **1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 16** and **17** showed better antibacterial potential than both antibiotics tested, and all compounds exhibited higher activity then ampicillin (Table 1).

A structure-activity relationship study revealed that the presence of a nitro group in general is favorable for antibacterial activity. At the same time the position and not only the nature of the substituent on the

Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr

Table.1. Antibacterial potential of tested compounds (MIC/MBC × 10^{-2} microM) and calculated Free binding Energy (Kcal/mol) to *E.coli* MurB (2Q85) and *E.coli* MurA (3KR6)



			MIC/MBC × 10^{-2} microM								Free Binding Energy (Kcal/mol)	
Com/ds	R		***S.a.***	***B.c.***	***L.m.***	***M.f.***	***S.t.***	***En.cl.***	***P.a.***	***E.c.***	**2Q85**	**3KR6**
8	4-NO_2	MIC	**1.65**	**1.65**	**3.30**	**2.20**	**1.65**	**1.80**	**2.20**	**2.20**	**-13.55**	
		MBC	3.30	3.30	6.60	3.30	3.30	6.60	4.40	3.30		
6	2-NO_2	MIC	**3.22**	3.22	**3.22**	**3.22**	**3.22**	**3.22**	**3.22**	**3.22**	**-12.85**	
		MBC	6.44	6.44	6.44	6.44	6.44	6.44	6.44	6.44		
7	3-NO_2	MIC	**4.18**	2.14	**1.70**	**4.18**	**2.14**	**4.18**	**2.14**	**4.18**	**-12.46**	**-9.35**
		MBC	8.36	4.20	2.14	8.36	4.28	8.16	4.28	8.16		
11	2,6-di-F	MIC	**4.36**	**4.36**	**2.18**	**4.36**	**4.36**	**2.18**	**4.36**	**4.36**	**-9.87**	
		MBC	8.72	8.72	4.36	8.72	8.72	4.36	8.72	8.72		
1	H	MIC	**4.73**	**9.47**	**9.47**	**9.47**	**2.36**	**9.47**	**9.47**	**9.47**	**-9.74**	
		MBC	9.47	18.94	18.94	18.94	4.72	18.94	18.94	18.94		
2	4-OH	MIC	**6.80**	**3.40**	**28.40**	**14.20**	**3.40**	**3.40**	**3.40**	**9.10**	**-9.01**	
		MBC	14.20	6.80	56.80	56.80	6.80	14.20	6.80	14.20		
3	4-OMe	MIC	**4.48**	**9.95**	**4.48**	**9.95**	**4.48**	**9.95**	**9.95**	**9.95**	**-8.72**	
		MBC	8.96	19.90	8.96	19.90	8.96	19.90	19.90	19.90		
17	2,6-di-Cl	MIC	**4.07**	**4.07**	**2.14**	**4.07**	**4.07**	**2.14**	**4.07**	**4.07**	**-8.27**	**-10.44**
		MBC	4.06	32.58	32.58	32.58	16.30	32.58	32.58	32.58		
5	4-Me	MIC	**11.46**	**11.46**	**5.93**	**11.46**	**5.93**	**11.46**	**11.46**	**11.46**	**-8.22**	
		MBC	22.72	22.72	11.46	22.72	11.46	22.72	22.72	22.72		
4	4-OH, 3-OMe	MIC	**3.20**	**3.20**	**13.40**	**13.40**	**3.20**	**3.20**	**3.20**	**13.40**	**-8.01**	
		MBC	26.80	13.40	53.60	26.80	13.40	13.40	13.40	26.80		
9	4-$N(Me)_2$	MIC	**13.91**	**13.91**	**6.95**	**13.91**	**6.95**	**13.91**	**6.95**	**13.91**	**-7.22**	
		MBC	27.82	27.82	13.90	27.82	13.90	27.82	13.90	27.82		
16	2,4-di-Cl	MIC	**2.03**	**16.29**	**16.29**	**16.29**	**8.15**	**16.29**	**16.29**	**16.29**	**-7.15**	
		MBC	4.06	32.58	32.58	32.58	16.30	32.58	32.58	32.58		
12	4-Br	MIC	**6.00**	**1.40**	**25.00**	**25.00**	**1.40**	**6.00**	**6.00**	**8.00**	**-7.03**	**-8.51**
		MBC	25.00	6.00	50.00	50.00	6.00	12.50	12.50	12.50		
15	2,3-di-Cl	MIC	**24.43**	**24.43**	**24.43**	**24.43**	**12.21**	**24.43**	**12.21**	**24.43**	**-6.88**	**-5.1**
		MBC	48.86	48.86	48.86	48.86	24.42	48.86	24.42	48.86		
13	3-Cl	MIC	**8.80**	**6.60**	**13.70**	**6.60**	**6.60**	**13.70**	**6.60**	**6.60**	**-6.74**	**-8.29**
		MBC	13.70	13.70	54.80	27.40	13.70	27.40	13.70	27.40		
10	4-F	MIC	**14.75**	**29.50**	**29.50**	**29.50**	**29.5**	**14.75**	**29.50**	**29.50**	**-6.25**	
		MBC	29.5	59.00	59.00	59.00	59.00	29.50	59.00	59.00		
14	4-Cl	MIC	**13.70**	**13.70**	**27.40**	**32.80**	**3.30**	**13.70**	**3.30**	**27.40**	**-5.74**	
		MBC	54.80	27.40	82.20	54.80	6.60	27.40	6.60	54.80		
Amp.		MIC	24.80	24.8	37.20	24.80	24.80	24.80	74.40	37.20		
		MBC	37.20	37.2	74.40	37.20	49.20	37.20	124.0	49.20		
Strept.		MIC	17.20	4.30	25.80	8.60	17.20	4.30	17.20	17.20		
		MBC	34.40	8.60	51.60	17.20	34.40	8.60	34.40	34.40		

B.c.-B.cereus, M.f.-M.flavus, S.a.-S.aurues, l.m.L.monocytogenes, E.c.-E.coli, En.c.-En.cloacae, P.a.-P.aeruginosa, S.t.-S.typhimurium. Relative standard deviations were all < 2.0. Amp.: Ampicillin, Strept.: Streptomycin. Colors for MIC: pink:1.40-5.00, orange:5.01-10.00, green:10.01-20.00, light blue:20.01-25.00, dark blue:>25.00. Colors for Free Binding Energy: pink:<-9.80, orange: -9.79 to -8.50, green: -8.49 to -7.00, light blue:-6.99 to 6.50, dark blue:-6.49 to -5.50, grey:>-5.50

Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr

Table 2. Antibacterial potential of tested compounds toward resistant strains of bacteria (MIC/MBC × 10^{-2} microM).

Comp.	R		*MRSA*	*P.a.*	*E.c.*
1	H	MIC	4.73	4.73	2.36
		MBC	8.14	8.14	4.72
3	4-OMe	MIC	11.06	11.06	5.53
		MBC	22.12	22.12	11.06
5	4-Me	MIC	11.47	11.47	11.47
		MBC	22.94	22.94	22.94
6	2-NO_2	MIC	17.50	17.50	17.50
		MBC	35.00	35.00	35.00
7	3-NO_2	MIC	10.7	5.35	5.35
		MBC	21.4	10.7	10.7
8	4-NO_2	MIC	0.80	0.80	0.60
		MBC	1.65	0.80	0.80
9	$N(CH_3)_2$	MIC	15.05	15.05	7.51
		MBC	30.10	30.10	15.02
10	4-F	MIC	29.54	29.54	29.54
		MBC	59.08	59.08	59.08
11	2,6-di-F	MIC	39.30	39.30	39.30
		MBC	78.60	78.60	78.60
15	2,3-di-Cl	MIC	18.32	18.32	18.32
		MBC	36.64	8.14	36.64
16	2,4-di-Cl	MIC	8.14	16.28	2.03
		MBC	16.28	16,28	4.06
17	2,6-di-Cl	MIC	6.11	6.11	3.05
		MBC	12.22	12.22	6.1
Ampicillin		MIC	-	124.0	124.0
		MBC	-	-	-
Streptomycin		MIC	25.80	12.90	25.80
		MBC	-	25.80	51.60

MRSA-Methicillin resistant Staphylococcus aureus, P.a-Pseudomonas aeruginosa, E.c.- Escherichia coli.

Relative standard deviations were all < 1.2

benzene ring also influences the activity. Thus presence of a substituent at the 4-position is most favorable followed by 2-NO_2 (**6**) and 3-NO_2 (**7**) respectively. Introduction to the unsubstituted derivative (**1**) of OH (**2**) and OMe (**3**) groups in position 4 of the benzene ring led to a slight decrease in activity of these derivatives, while introduction of a 4-Me (**5**) group decreased the activity further. Introduction of a methoxy group at position 3 of compound (**2**) also had a negative effect on the activity (compound **4**). As far as halogen derivatives are concerned, in general, monosubstitution is not favorable, for fluoro-, bromo- or chloro- derivatives. However, 2,6-fluoro- (**11**) substitution exhibited a completely different effect on antibacterial activity. Among the dichloro- substituted derivatives the order of activity was found to be 2,6-Cl > 2,4-Cl > 2,3-Cl, showing that in case of dihalogen substitution, position 6 is more favorable compared to others.

The antibacterial capacity of the tested compounds towards resistant strains of bacteria (MRSA, *Pseudomonas aerugniosa* and *Escherichia coli*) and follows the order: **8 > 1 > 17 > 16 > 7 > 3 > 5 > 9 > 15 > 6 > 10 > 11** (Table 2).

Compound (**8**) again showed the best antibacterial potential, the same as against ATCC strains, with MIC at 0.60-0.80 × 10^{-2} microM and MBC at 0.80-1.65 × 10^{-2} microM, respectively

The lowest antibacterial activity is observed for compound (**11**), with MIC at 39.30 × 10^{-2} microM and MBC at 78.60 × 10^{-2} microM. All tested compounds expressed higher antibacterial potential than both antibiotics tested (Table 2).

The most sensitive bacterial species is *S. typhiumurium,* while *M. flavus* was the most resistant among ATCC strains, and MRSA was the most resistant among all tested bacteria (ATCC and clinical isolates).

Ampicillin exhibited an inhibitory potential at 24.8-74.4 × 10^{-2} microM and bactericidal at 37.2-124.0 × 10^{-2} microM but no bacteriostatic or bactericidal effects were observed against MRSA. Streptomycin possessed MIC at 4.30-17.20 × 10^{-2} microM and MBC at 8.60-34.40 × 10^{-2} microM, but no bactericidal effect was observed against MRSA.

Structure-activity relationship studies revealed that the presence of nitro group in position 4 of benzene ring (**8**) favours the activity against resistant strains. But in general substitution in benzene ring of the parent compound (**1**) decrease the activity towards resistant strains. It was observed that this depends not only on the nature of substituent but also on its position in benzene ring. Thus, 2,6-dichloro- substitution is more favourable than 2,4-dichloro- and much more favourable than 2,3-dichloro-. The same was found for nitro derivatives (4-NO_2 >> 2-NO_2), while fluoro- derivatives appeared to be the less active against resistant bacteria strains.

The antifungal potential of tested compounds is presented in Table 3 and follows the order: **8 > 1 > 4 > 12 > 17 > 3 > 7 > 10 > 16 > 13 > 14 > 2 > 9 > 15 > 5 > 11 > 6.** Compound (**8**) showed the best antifungal potential, as in the case of antibacterial potential. Inhibitory activity was achieved at 0.60-1.65 × 10^{-2} microM, and fungicidal at 0.80-3.30 × 10^{-2} microM. The lowest antifungal effect was observed for compound (**6**), with MIC and MFC at 32.11-48.17 × 10^{-2} microM and 64.22-96.34 × 10^{-2} microM, respectively.

Ketoconazole showed antifungal potential at MIC 37.60-380.00 × 10^{-2} and MFC 94.00-475.00 × 10^{-2} microM respectively, while bifonazole showed MIC at 32.00-64.00 × 10^{-2} and MFC at 48.30-80.00 × 10^{-2} microM respectively. From the results obtained it can be seen that all tested compounds exhibited higher antifungal potential than both antimycotics tested (Table 3.).

The most sensitive fungal species are *T. viride* and *A. ochraceus* while the *A. fumigatus* appeared to be the most resistant one (Table 3).

Structure-activity relationship studies revealed that, while the presence of nitro group in position 4 (**8**) of benzene ring is beneficial for antifungal activity, 2-NO_2 (**6**) substitution is deleterious while the 3-NO_2 derivative (**7**) exhibited moderate activity. Derivative (**1**) lacking any substituent on the benzene ring appeared to be the second most active compound against fungi, while introduction of other substituents led to decreases in activity. Thus, introduction of bromine into position 4 of the benzene ring resulted in the third most active compound (**12**) while introduction of a 4-chloro- substituent remarkably decreased the activity. Among the dichloro- derivatives a beneficial effect was observed for 2,6-disubstitution compared to 2,4- and 2,3-substitution.

As far as the monochloro-derivatives are concerned it seems that compound (**13**) substituted in position 3 is a little more active than that substituted in position 4 (**14**). 4-Fluoro- substitution appeared to be more favourable than 2,6-difluoro- substitution. Again, no correlation of activity with lipophilicity was observed.

Summarising the results above, it is apparent that compound (**8**) possesses the highest antibacterial and antifungal potential among all compounds synthesized.

Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr

Table 3. Antifungal potential of tested compounds (MIC/MFC × 10^{-2} microM).

Comp.	R		*A.fum.*	*A.v.*	*A.o.*	*A.n.*	*T.v.*	*P.f.*	*P.o.*	*C.a.*	*P.v.c*
1	H	MIC	4,75	9,50	4,75	9,50	4,75	4,75	4,75		9.500
		MFC	9,50	19,00	9,50	19,00	9,50	9,50	9,50		19.00
2	4-OH	MIC	14.2	7.1	7.1	14.2	7.1	7.1	14.2	28.4	
		MFC	28.4	14.2	14.2	28.4	14.2	28.4	28.4	56.8	
3	4-OMe	MIC	9,98	9,98	9,98	17,86	17,86	9,98	9,98		9.98
		MFC	19,96	19,96	19,96	35,72	35,72	19,96	19,96		19.96
4	4-OH, 3-OMe	MIC	13.3	3.3	1.7	6.6	1.7	6.6	6.6	13.3	
		MFC	26.6	13.3	13.3	26.6	6.6	13.3	13.3	26.6	
5	4-Me	MIC	36,69	18,35	18,35	18,35	36,69	18,35	18,35		18.35
		MFC	73,38	36,70	36,70	36,70	73,38	36,70	36,70		36.70
6	2-NO_2	MIC	32,11	32,11	32,11	48,17	32,11	48,17	32,11		32.11
		MFC	64,22	64,22	64,22	96,34	64,22	96,34	64,22		64.22
7	3-NO_2	MIC	10,70	10,70	10,70	21,40	5,35	10,70	21,40		10.70
		MFC	21,40	21,40	21,40	21,40	10,70	21,40	42,80		21.40
8	4-NO_2	MIC	0.80	0.80	1.23	1.65	0.60	1.23	0.80		1.65
		MFC	1.60	1.60	2.46	3.30	0.80	1.65	1.60		3.30
9	$N(CH_3)_2$	MIC	30,10	15,05	15,05	15,05	30,10	15,05	15,05		30.10
		MFC	60,20	30,10	30,10	30,10	60,20	30,10	30,10		60.20
10	4-F	MIC	9,09	9,09	9,09	18,18	18,18	9,09	9,09		18.18
		MFC	18,18	18,18	18,18	36,36	36,36	18,18	18,18		36.36
11	2,6-di-F	MIC	39,3	39,3	39,3	39,3	39,3	39,3	39,3		39.30
		MFC	78,6	78,6	78,6	78,6	78,6	78,6	78,6		78.60
12	4-Br	MIC	12.5	6.25	1.56	6.25	3.12	12.5	6.25	20.0	
		MFC	25.0	12.5	3.12	12.5	12.5	25.0	12.5	25.0	
13	3-Cl	MIC	13.7	6.8	6.8	13.7	6.8	13.7	6.8	27.4	
		MFC	27.4	13.7	13.7	27.4	13.7	27.4	13.7	54.7	
14	4-Cl	MIC	13.7	6.8	6.8	13.7	6.8	13.7	13.7	27.4	
		MFC	27.4	13.7	13.7	39.4	13.7	27.4	27.4	54.7	
15	2,3-di-Cl	MIC	16,29	16,29	16,29	16,29	16,29	32,58	16,29		16.29
		MFC	32,58	32,58	32,58	32,58	32,58	65,16	32,58		32.58
16	2,4-di-Cl	MIC	8,14	16,28	8,14	8,14	8,14	16,28	16,28		8.14
		MFC	16,28	32,56	16,28	16,28	16,28	32,56	32,56		16.28
17	2,6-di-Cl	MIC	6,10	6,10	9,20	6,10	12,20	6,10	6,10		12.20
		MFC	12,20	12,20	18,40	12,20	24,40	12,20	12,20		24.40
Ketoconazole		MIC	38.0	285.0	38.0	38.0	475.0	38.0	380.0	37.6	37.60
		MFC	95.0	380.0	95.0	95.0	570.0	95.0	380.0	94.0	94.00
Bifonazole		MIC	48.0	48.0	48.0	48.0	64.0	64.0	48.0	32.2	32.20
		MFC	64.0	64.0	80.0	64.0	80.0	80.0	64.0	48.3	48.30

A.fum.-A.fumigatus, A.v.-A.versicolor, A.o.-A.ochraceus, A.n.-A.niger, T.v.-T.viride, P.f.-P.funiculosum, P.o.-P.ochrochloron, C.a.-C.albicans, P.v.c.-P.cyclpoium var verucosum.

Relative standard deviations were all < 2.20, except for antimycotics < 4.50

6. Docking studies

6.1. Docking to E.coli MurB

Based on the literature, thiazolidinones are known to act via MurB inhibition.[39] Thus, theoretical binding studies of the compounds at the active site of E. coli MurB (PDB code: 2Q85) were performed. For the determination of the docking box center, the oxygen of -OH of the side chain of Ser228 was used. Ser228 is a catalytic residue that plays an important role for the activity of the enzyme because it takes part in the proton transfer to an enol intermediate that is formed during the second reduction step.[69] The Free binding energy of the compounds correlates well with the biological activity to *E.coli* and relatively well with the activity to other microorganisms (Table 1).

The interacting amino acids of all compounds with *E.coli* MurB are shown at Table 4.

The docking pose of the most active compound (**8**) showed four favorable hydrogen bonds, the first two between the oxygen atom of the nitro group of (**8**) and the hydrogens of the side chain of Gln119 and Arg326 (distance 2.24 Å and 2.29 Å respectively), one between the nitrogen of the thiazole ring of (**8**) and the hydrogen of the side chain of Arg213 (distance 2.37 Å) and the last between the oxygen atom of the thiazole carboxyl group of (**8**) and the hydrogen of the side chain of Ser228 (distance 2.01 Å). The benzene core shows hydrophobic interactions with Asn50, Pro110, Ala226, Glu324, Ile121, Ile109 and Ser49 (Figure 1), while the adamantane ring forms hydrophobic contacts with Lys216, Gln287, Met212, Tyr124, Tyr189 and Leu217 (Figure 1).

The least active compound **14** showed only a few hydrophobic interactions with the residues Ile121, Ile118, Tyr124, Pro110, Leu217 and Tyr18 (Figure 2). Furthermore steric interactions are observed that destabilize the complex and decrease dramatically the inhibitory action of the compound

As observed by the results of Table 1, the compounds exhibit similar activity against different bacteria in many cases. Structural and sequence similarity at the active site of MurB can explain this observation. Structural alignment of *E.coli* MurB

Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr

(2Q85) with the MurB enzymes of *Pseudomonas aeruginosa* (4JAY), *Listeria monocytogenes* (3TX1) and *Staphylococcus aureus* (1HSK) indicated 43.88% identity with 4JAY, 22.59% with 3TX1 and 25.68% with 1HSK with conservation of important aminoacids such as Gly122, Tyr 124, Arg213 and Ser218 in all cases. Structural and amino acid alignments are shown in supplementary material, Fig 4S-6S.

Table 4. Calculated Binding affinity score and important amino acids for interaction with *E. Coli* Mur B 2Q85

N	Free binding energy	Binding affinity	Hb	Residues
1	-9.74	-27.13	3	Gly122, Ser228, Asn232
2	-9.01	-25.11	3	Tyr124, Tyr189, Ser228
3	-8.72	-25.55	2	Arg213, Ser228
4	-8.01	-22.79	2	Gly122, Ser228
5	-8.22	-23.47	2	Arg213, Ser228
6	-12.85	-36.79	4	Tyr124, Arg158, Arg213, Ser228
7	-12.46	-37.55	4	Gly122, Tyr124, Tyr189, Ser228
8	-13.55	-39.11	4	Gln119, Arg326, Arg213, Ser228
9	-7.22	-21.58	2	Arg213, Ser228
10	-6.25	-18.55	1	Gly122
11	-9.87	-29.16	3	Arg326, Arg213, Ser228
12	-7.03	-20.42	1	Tyr189
13	-6.74	-19.72	1	Tyr189
14	-5.74	-14.88	-	-
15	-6.88	-20.13	1	Gly122
16	-7.15	-21.49	2	Arg213, Ser228
17	-8.27	-23.84	2	Arg213, Ser228

Hb: Hydrogen bond interactions

Docking analysis of the most active compound 8 to the structures 4JAY, 3TX1 and 1HSK revealed increased probability of stable binding with the enzymes with Estimated Free binding Energies varying between -13.40 and -14.55 kcal/mole, Hydrogen bond formation, polar, hydrophobic and pi-pi interactions with amino acids of the active site stabilize the complexes (Table 5, Figures 4S, 5S of the supplementary files).

The docking of compound 8 at the MurB enzyme of *Pseudomonas aeruginosa* (4JAY) is shown at the Figure 3. Two hydrogen bonds are formed with Gln297 and Gln298. Hydrophobic interactions between Leu228 and the adamantine moiety are also observed, while Trp277 participates in pi-pi interactions with the phenyl ring of the compound (Figure 3).

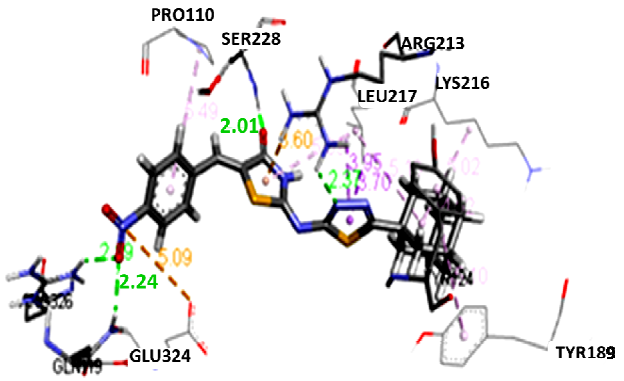


Figure 1. (a1-2) Docked conformation of the most active compound **8** in *E.coli* MurB (**2Q85**).

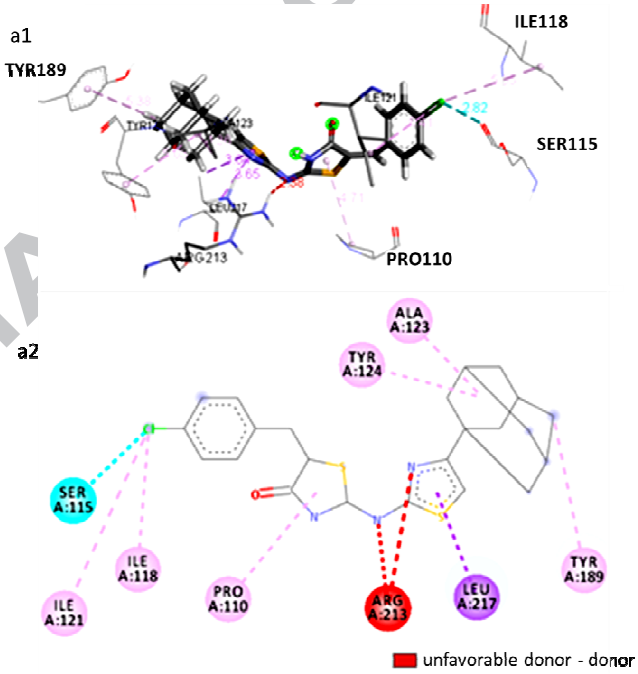


Figure 2. (a1-2) Docked conformation of the less active compound **14** in *E.coli* MurB(**2Q85**).

Table 5: Calculated Free Binding Energy and important amino acids for interaction of compound 8 with MurB *Pseudomonas aeruginosa, P.a.,* (4JAY), *Listeria monocytogenes, L.m.,* (3TX1) and *Staphylococcus aureus, S.a.,* (1HSK).

Microorganism (structure)	Free binding energy (kcal/mol)	Hb	Residues
***P.a.* (4JAY)**	-14.55	2	Gln297, Gln298, Leu228, Trp277
***L.m.* (3TX1)**	-13.40	2	Ser220, Arg224, Tyr169, His253
***S.a.* (1HSK)**	-13.58	1	Arg242, Tyr187, Phr247

Hb: Hydrogen bond interactions

Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr

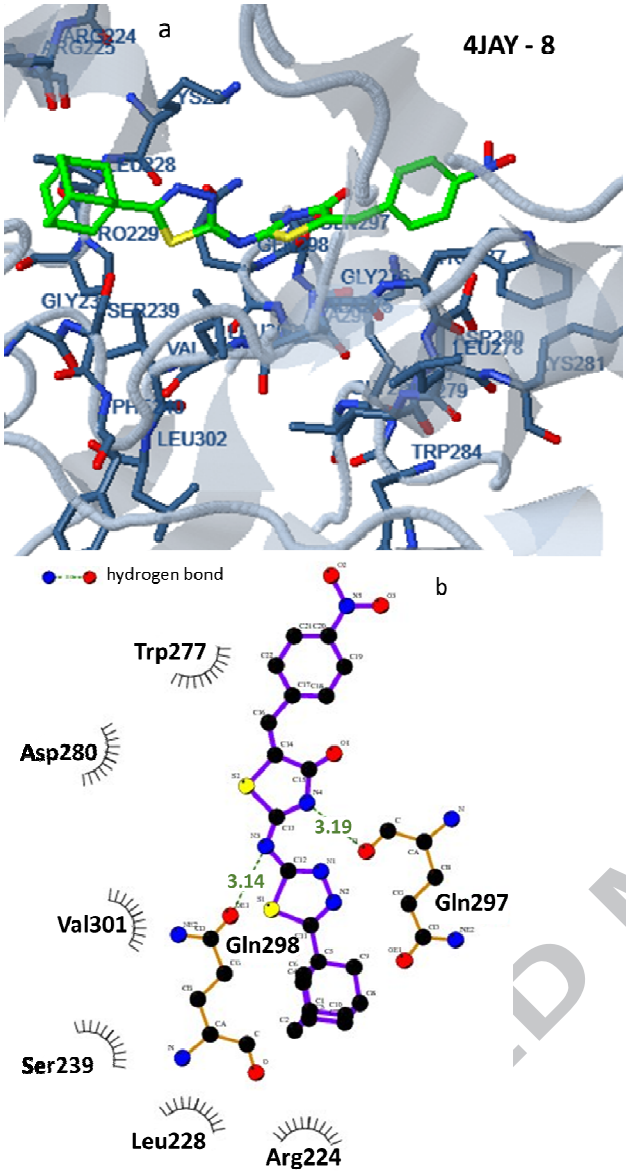


Figure 3. Docking of compound **8** to the MurB of *Pseudomonas aeruginosa* (4JAY)

The docking of compound 8 to the MurB of t *Listeria monocytogenes* (3TX1) and of *Staphylococcus aureus* (1HSK) are shown in Figure 7S and 8S of the supplementary material.

The low values of the calculated Free Energy for Binding of the most active compound, **8**, to MurB enzyme of *E.c., P.a., L.m. and S.a.* indicate that inhibition of MurB may explain the antibacterial activity in all tested bacteria.

The form of correlation between in vitro antibacterial activity to *E.coli* (MIC) and the Free binding Energy to *E.coli* MurB was further investigated using MyCurveFit online program. The application exported a curve fit with R^2=0.692, aR^2=0.5891 and p=0.00441 which corresponds to a fourth order polynomial regression presented in Figure 4A. Observation of the curve, as well as the Table 1, revealed that three compounds, **17, 12** and **13,** were mostly responsible for the poor correlation. All three compounds exhibited better *in vitro* activity than expected according to their calculated Free binding Energy.

When these three compounds were excluded, application of MyCurveFit revealed a correlation that can follow an exponential equation with R^2=0.9234, aR^2=0.9025 and p=0.000001993 (Figure 4B).

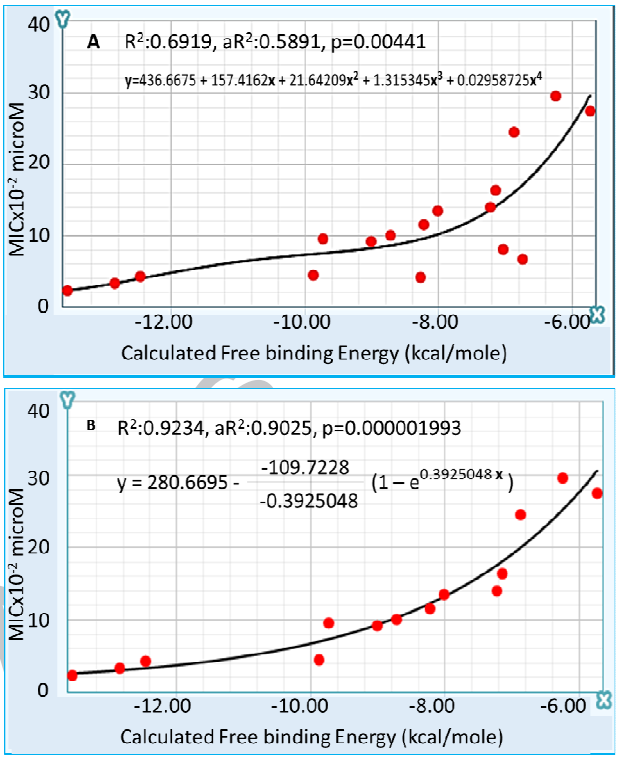


Figure 4. Best curves describing the correlation between in vitro anti-bacterial properties (MICx10^{-2} microM) and calculated Free binding Energy to *E.coli* 2Q85 of A) all the compounds, B) of the compounds after removing the results of the compounds 12, 13 and 17.

The observation supported the assumption that these three compounds may act via a different mechanism. Since MurA is also a drug target for antibacterial agents, docking analysis of these three compounds **17, 12** and **13,** and of compounds **7** and **15** to *E.coli* MurA (structure 3KR6) was performed. The results are also shown in Table 1. It can easily be seen that the calculated Free binding Energy of the compounds **17, 12** and **13** to *E.coli* MurA correlates well with their *in vitro* activity. The calculated Free binding Energy to MurA of the compounds **7** and **15** which were chosen for comparison reasons was low compared to the *in vitro* activity, indicating that these compounds probably act as MurB inhibitors. Total lack of activity against MurA was predicted for compound **15** with calculated free binding Energy>-5.5 kcal/mole.

The results of the docking studies and the reference drug (ketoconazole) are given in the Table 6.

As shown in Table 6, interactions with Arg120 of *E.coli* MurA were observed in all cases with the exception of compound **15** for which no interactions with amino acids of the active site were observed.

Docking of the most potent MurA inhibitor, compound **17** is shown in figure 5. The compound is oriented with the phenyl ring in vicinity to Gly114, where fosfomycin is also placed. Hydrogen bond

Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr

interactions with Arg91 and Arg120 stabilize the complex while Arg397 and Phe328 participate in ion-pi and pi-pi interactions with the phenyl group and thiazolyl ring respectively.

Table 6: Calculated Free Binding Energy, Binding affinity score and important amino acids for interaction with *E. coli* MurA: 3KR6

Comp.	Free binding energy	Binding affinity score	Hb	Residues
7	-9.35	-32.76	2	Arg120, Lys22
12	-8.51	-31.38	2	Arg120
13	-8.29	-30.24	1	Arg120
15	-5.10	-16.45	-	-
17	-10.44	-36.23	2	Arg120, Arg91

Hb: Hydrogen bond interactions

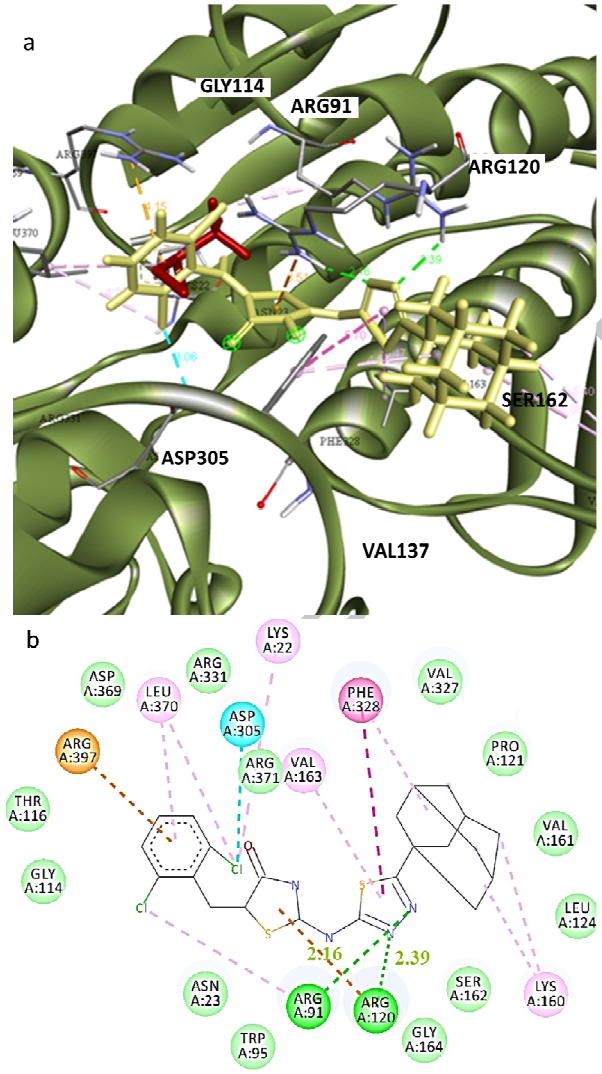


Figure 5 a. Docking of compound 17 (yellow) to E.Coli MurA (3KR6). Fosfomycin (red) is also shown for comparison. b. Interactions of the compound with amino acids of the binding site. Hydrogen bonds are shown in green. Pi-ion interactions are shown in orange and pi-pi interaction in dark pink.

6.2. Docking to lanosterol 14α-demethylase of C. albicans (CYP51)

Most antifungals target to the inhibition of the biosynthesis of ergosterol, a main component of the cytoplasmic membrane of these microorganisms. Several categories of compounds have been found to inhibit different enzymes of this pathway such as the squalene epoxidase, ERG1, (allylamides and thiocarbamates), ERG2 and ERG24-27 (morpholines), ERG4 (polyenes), 14alanosterol demethylase, ERG11 (azoles)[69]. Moreover, according to previous research, several thiazolyl, thiazolydinone derivatives have exhibited increased probability to act as 14alanosterol demethylase inhibitors[70]. Based on these, *Candida albicans* ERG11 enzyme (CYP51), available in the PDB database, PDB ID:5v5z, was chosen for Docking analysis, as a first efford to explore the probable mechanism of anti-fungal activity of the compounds. *C. albicans* Dihydrofolate reductase (PDB ID: 4HOF) was also used for docking analysis.[71]

Table 7: Estimated binding Energy (kcal/mol) to Dihydrofolate reductase (DHFR, PDB ID:4HOF) and lanosterol 14alpha-demethylase (CYP51, PDB ID:5V5Z) of *Candida Albicans* and important amino acid residues for interaction of the compounds with CYP51 of *C. albicans*, 5V5Z

Comp.	Free binding Energy (kcal/mol) DHFR 4HOF	Free binding Energy (kcal/mol) CYP51 5V5Z	Hb	Residues of CYP51 5V5Z
1	-8.50	-13.54	2	Tyr118, Tyr132
2	-5.82	-8.69	1	Tyr132
3	-7.55	-10.55	1	Tyr132
4	-8.24	-12.47	2	Tyr118, Ser312
5	-5.14	-7.21	-	-
6	-4.21	-6.52	-	-
7	-7.19	-10.21	2	Tyr95, Gly472
8	-8.74	-14.85	1	Tyr118
9	-6.01	-8.13	1	Ser312
10	-7.15	-9.84	1	Gly472
11	-5.11	-7.02	-	-
12	-8.13	-11.95	2	Tyr118, Tyr311
13	-5.92	-8.77	1	Tyr311
14	-6.76	-8.13	-	-
15	-5.18	-7.33	-	-
16	-7.12	-9.62	1	Tyr132
17	-7.88	-11.23	2	Tyr118, Tyr132
Ketoc.	-6.75	-8.23	1	Tyr64

Ketoc.: Ketoconazol, Hb: Hydrogen bond interactions

Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr

The calculated Free Binding Energy to CYP51 varied between -6.52 kcal/mole for compound **6** which exhibited the lowest in vitro activity and -14.85 kcal/mole for the most active compound **8**. Interestingly, the calculated Free Energy follows the same order as the experimentally measured MIC, **8< 1 < 4 < 12 < 17 < 3 < 7 < 10 < 16 < 13 < 2 < 14 = 9 < 15 < 5 < 11 < 6,** with a reverse in the positions of compounds 2 and 14, which strongly supports the suggestion that CYP51 inhibition is involved in the anti-fungal action of the compounds. Higher Free binding Energies (low inhibitory action) were calculated for docking of the compounds to the human enzyme.

According to the docking results (Table 7), the synthesized compound as well as the reference compound, ketoconazole, bind in a similar way to $CYP51_{Ca.}$ For compound **8** a hydrogen bond interaction was detected between the H of the side chain of Tyr118 of $CYP51_{Ca}$ and S of the thiazole ring of the compound(distance 2.76 Å). Moreover, hydrophobic interactions between Tyr132, Ile131 and Leu376 and the adamantane ring of the compound **8** were detected. During the docking study, the heme group of the protein exhibited positive interactions with the heterocyclic rings of the compound 8, as well as with the benzene ring of ketoconazole (figure 6)

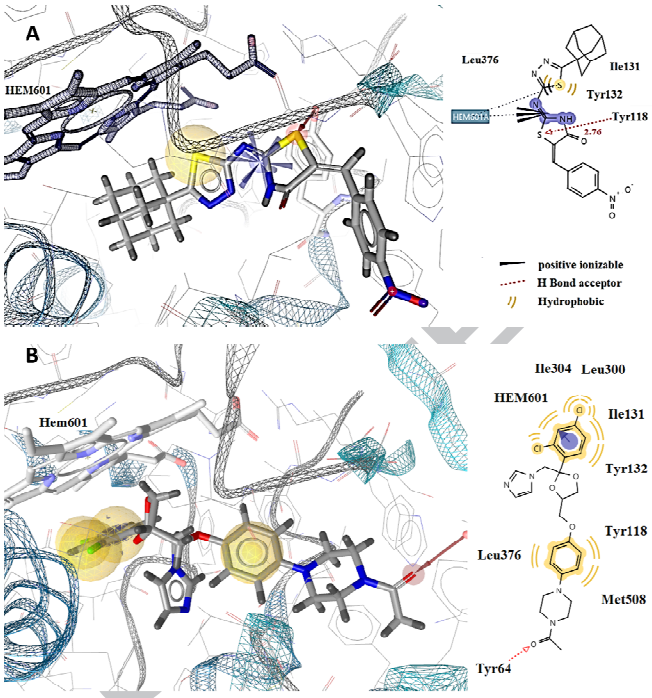


Figure 6: Docked conformation of the most active compound **8 (A)** and ketoconazole **(B)** in lanosterol 14alpha-demethylase of *C. albicans* ($CYP51_{ca}$) (5V5Z).

The calculated Free Energies for binding to the tetrahydrofolate reductase of *Candida Albicans* were indicating potentially active molecules for most of the compounds with the exception of compounds 6, 5, 11 and 15 for which the calculated Free Energy was lower than -5.5 kcal/mole. The calculated Free Binding Energies varied between -4.21 kcal/mole for the less active compound **6** and -8.74 kcal/mole for the most active compound **8**. Most interestingly, the calculated Free binding Energy for the eleven more active compounds also follows the order of the experimentally measured biological activity.

7. Conclusion

The range of seventeen newly designed and synthesized **2**{[5-(adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-arylidene-1,3-thiazolidin-4-ones exhibited a remarkable inhibition of the growth of a wide spectrum of Gram-positive, Gram-negative bacteria and fungi. All compounds except of (**10**) and (**15**) exhibited better or comparable antibacterial activity compared to the reference drug ampicillin (up to 15 fold). Furthermore, some of the compounds showed better or comparable potency compared to streptomycin with the most potent among them being compound **8** (15 and 7 fold more active than ampicillin and streptomycin respectively)**.** It was observed that, among the Gram-negative bacteria, the most sensitive to the tested compounds was *S. typhimirium*, while *E.coli* was the most resistant one. Regarding the Gram-positive bacteria, the most sensitive one was *S. aureus,* while *L .monocytogenes* was found to be the most resistant bacterium.

As far as fungi were concerned, the tested compounds possess excellent activity against all the fungal species tested, being 3-115 times more active than ketoconazole and 1.7-37 times more active than bifonazole with the exception of compounds (**6**) and (**11**). The most promising was compound (**8**), followed by the parent structure (**1**).

The most sensitive fungi to compounds tested were found to be *T. viride* and *A. ochraceus* while the *A. fumigatus* appeared to be the most resistant one It can be observed that the growth of both Gram-negative and Gram-positive bacteria and fungi responded differently to the tested compounds, which indicates that different substituents may lead to different modes of action or that the metabolism of some bacteria/fungi was better able to overcome the effect of the compounds or adapt to it.

Docking analysis to *E.coli* MurB and MurA indicated a probable involvement of MurB inhibition in the anti-bacterial mechanism of most compounds and a probable involvement of MurA inhibition at the mechanism of action of compounds **12, 13** and **17**.

Docking analysis to 14a-lanosterol demethylase (CYP51) and tetrahydrofolate reductase of *Candida albicans* indicated a probable implication of CYP51 reductase at the anti-fungal activity of the compounds and a secondary involvement of dihydrofolate reductase inhibition at the mechanism of action of the most active compounds.

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Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr

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Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr

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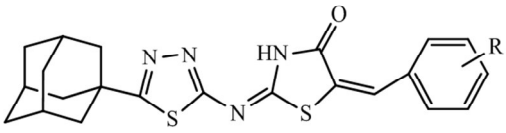
Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr

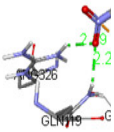
Highlights

1. Novel adamantanyl thiadiazolyl thiazolidinones with antimicrobial activity.
2. Molecular docking studies on *E.coli* MurB and MurA enzymes
3. Docking studies on dihydrofolate reductase and CYP51 enzymes of *C. albicans*

Seventeen novel 2-{[5-(adamantan-1-yl)-1,3,4-thiadiazol-2-yl]-imino}-5-arylidene-1,3-thiazolidin-4-ones were designed, synthesized and evaluated for antimicrobial activity. All compounds were potent antimicrobial agents. According to molecular docking studies, inhibition of MurB and CYP 51 may be involved in the mechanism of antibacterial and antifungal activities of most of the compounds.



Best compound anti-bacterial activity: MIC 1.65-3.30 $\cdot 10^{-2}$ μM
anti-fungal activity: MIC 0.80-3-30 $\cdot 10^{-2}$ μM



Compound 8

Correspondent author: A.Geronikaki. Tel.: +302310997616; fax: +302310997612; e-mail: geronik@pharm.auth.gr