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ARTICLE TYPE

4-Thiazolidinone derivatives as potent antimicrobial agents: microwave-assisted synthesis, biological evaluation and docking studies

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As a part of our ongoing research in the development of new antimicrobials, herein, we report the synthesis of ten compounds which combine three bioactive moieties: thiazole, adamantane and 4-thiazolidinone. Evaluation of their antibacterial activity revealed that the newly synthesized compounds exhibited remarkable growth inhibition of a wide spectrum of Gram-positive bacteria, Gram-negative bacteria and fungi. The majority of the compounds displayed greater antibacterial activity than the reference drugs (ampicillin and streptomycin), while the antifungal activity was significantly higher than that of refereence drugs bifonazole and ketoconazole. Additionally, the title compounds were screened for HIV-1 reverse transcriptase inhibitory activity, showing no significant activity though. Moreover, docking studies were performed in order to explore possible binding modes at MurB protein of *S. aureus*.

1. Introduction

During the past decades, rapid scientific progress has been made in the treatment of infectious diseases. However, they still remain a serious and challenging health problem due to several factors which have led to the re-emergence of these diseases. Antibiotic resistance, population increase, international travel, migration, increase in the number of immune-suppressed patients, climate change are some of the factors that play a significant role in the battle against infectious diseases.[1-5]

In order to keep microorganisms' resistance under control, careful use of existing antimicrobial drugs and the design of novel drugs with different modes of action (e.g. linezolid [6-8]) are required.[9-12]

In continuation of our research on bioactive molecules,[13] we designed and synthesized a series of 2-aryl-3-[4-(adamantan-1-yl)thiazol-2-yl] thiazolidinone-4-one derivatives. We emphasized the strategy of combining three chemically different, but pharmacologically compatible moieties such as thiazole, thiazolidinone and adamantane within one frame.

The thiazole is one of the most intensively investigated classes[14-19] of aromatic five-membered heterocycles and its derivatives have a variety of medical applications such as bacteriostatics, antibiotics,[20] diuretics,[21] local anaesthetics,[22,23] anti-inflammatories,[24] analgesics,[25,26] antipyretics,[25] anti-HIV[27,28] etc. Thiazolidin-4-ones are known to possess a wide range of biological activities, such as antibacterial,[29-33] antifungal,[29-33] antiviral,[34-36] anti-inflammatory,[32] antitubercular[37-39] etc. Furthermore, adamantane is an interesting moiety in medicinal chemistry known for its antiviral,[40-43] antimicrobial,[44-46] anti-inflammatory[16,47] and anti-Alzheimer[48] activities.

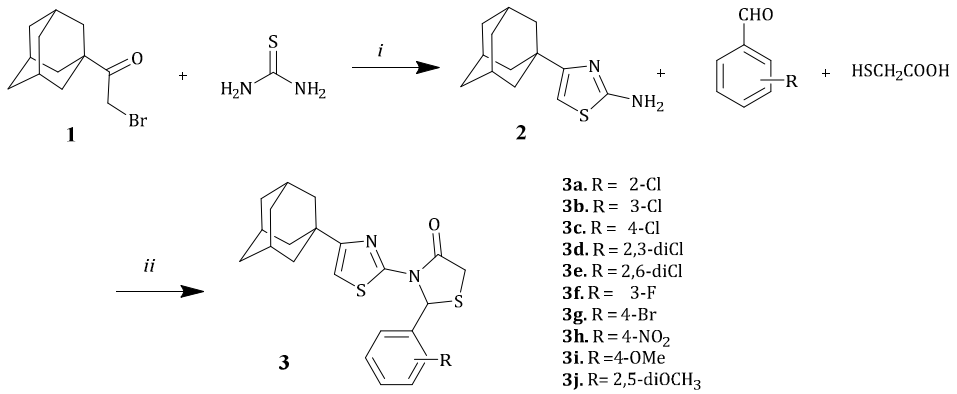
Therefore, in this paper we report the synthesis of ten 2-aryl-3-[4-(adamantan-1-yl)thiazol-2-yl] thiazolidinone-4-ones and their biological evaluation against a panel of Gram-positive bacteria, Gram-negative bacteria, fungi and HIV-1 reverse transcriptase. Furthermore, docking studies of the most active and one of the least active compounds were performed in order to explore their potential binding mode at MurB protein of *S.aureus*.

2. Results and discussion

2.1 Chemistry

The final compounds **3a-j** were prepared by one-pot three component reaction according to Scheme 1. 4-(Adamantan-1-yl)thiazol-2-amine (**2**) was prepared from 1-adamantyl bromomethyl ketone and thiourea according to the literature.[16] Subsequently, the reaction of the 4-(adamantan-1-yl)thiazol-2-amine with differently substituted aromatic aldehydes was performed in presence of mercaptoacetic acid in excess in absolute ethanol under microwave irradiation to deliver the final products (**3a-j**).[28,49] Overall, the reactions proceeded smoothly in moderate yields from 20−60%.

Structures and purity of the final compounds **3a-j** were confirmed by ^{1}H NMR, ^{13}C NMR, UPLC-MS and elemental analysis. In the ^{1}H NMR spectra, chemicals shifts of the final compounds appeared in the region of δ 1.45−1.96 (Ad), 3.98−4.04 (thiazolidinone, 5-H_AH_B), 4.15−4.39 (thiazolidinone, 5-H_AH_B), 6.62−6.79 (thiazolidinone, 2-H) and 6.74−8.19 (aromatic). In ^{13}C NMR spectra, chemicals shifts of the final compounds appeared in the region of δ 27.8−27.9 (adamantane), 32.3−33.9 (C-5, thiazolidinone), 35.7−35.8 (adamantane), 36.2−36.3 (adamantane), 55.2−56.3 (CH_3O), 59.0−63.0 (C-2, thiazolidinone), 105.4−163.2 (aromatic) and 170.1−170.6 (C=O).



Reaction conditions: *i)* isopropanol, r.t., 30min; *ii)* abs EtOH, m.w. irradiation, 110–130 °C, 20–60 min.

Scheme 1. Synthesis of the title compounds **3a-j**.

2.2 Biological evaluation

2.2.1 Antimicrobial activity

The synthesized compounds were assayed *in vitro* for their antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi obtained from ATCC (American Type of Culture Collection) or clinical/human isolates. Minimal inhibitory concentrations (MIC) that inhibited the growth of the tested microorganisms as well as minimal bactericidal/fungicidal concentrations (MBC/MFC) were determined using the microdilution method. The results of antimicrobial activity of the tested compounds **3a-j** and standard antibiotics/antimycotics against a panel of selected Gram-positive and Gram-negative bacteria and fungi are presented in Table 1 and Table 2, respectively.

The synthesized compounds showed antibacterial activity against all the tested bacteria, on different level though. MIC values were in a range of 0.9−43.8 μmol/ml x 10^{-2}, while MBCs were in a range of 1.53−86.0 μmol/ml x 10^{-2}. The antibacterial potential could be presented as following: **3d> 3c> 3i> 3h> 3b> 3a> 3f> 3g> 3j> 3e.**

The best antibacterial activity was obtained for compound **3d**, with MICs from 0.9−6.25 μmol/ml x 10^{-2} and MBCs from 1.53−12.5 μmol/ml x 10^{-2}. The lowest antibacterial activity among all compounds tested herein was obtained for **3e** with MICs from 21.5−43.0 μmol/ml x 10^{-2} and MBCs from 43.0−86.0 μmol/ml x 10^{-2}).

The most sensitive bacterial species was *S. typhimurium* with MIC values from 0.9−21.9 μmol/ml x 10^{-2} and MBC values from 1.53−43.8 μmol/ml x 10^{-2}, followed by *M. flavus* (MIC: 3.06−21.9 μmol/ml x 10^{-2} and MBC: 6.25−43.8 μmol/ml x 10^{-2}). *L. monocytogenes* was the most resistant species with inhibitory activity in a range of 1.53−43.8 μmol/ml x 10^{-2} and bactericidal at 12.5−86.0 μmol/ml x 10^{-2}, for almost all tested samples, apart from compounds **3a** and **3f**.

The standard drugs streptomycin and ampicillin, used as a positive controls, were also active against all bacteria. The range of MICs for streptomycin was 4.3−25.8 μmol/ml x 10^{-2} and MBCs was 8.6−51.6 μmol/ml x 10^{-2}, while ampicillin showed slightly lower antibacterial potential with MICs from 24.8−74.4 μmol/ml x 10^{-2} and MBC from 37.2−124.0 μmol/ml x 10^{-2}. It is worth noticing that compounds **3a-j** possessed very strong antibacterial activity, higher than that of ampicillin and streptomycin. For example, activity of compound **3d** against *S. aureus* was ~20-fold higher than that of ampicillin. Compounds **3e**, **3g** and **3j** were less potent than compounds **3a-3d**, **3f**, **3h** and **3i**. But still they showed higher activity than that of ampicillin and streptomycin against some bacteria.

The structure–activity relationship (SAR) indicated that shifting chlorine from position 4 (compound **3c**) to positions 3 (compound **3b**) or 2 (compound **3a**) did not affect significantly the activity, leading to slightly less active compounds, however still comparable. Introduction of a second chlorine on the phenyl ring led to enhanced activity in case of the 2,3-diCl-substituted compound (**3d**) which exhibited the best antimicrobial profile. However, diCl-substitution at 2 and 6 positions of phenyl ring (compound **3e**) decreased dramatically the antimicrobial activity. On the other hand, introduction of a methoxy group (compound **3i**) or nitro group (compound **3h**) at position 4 of the phenyl ring preserved the antimicrobial activity. In case of 4-F substituted phenyl, the activity increased against most of the species apart from *B. cereus* and *S. aureus* that dropped significantly. Introduction of bromine at position 4 (compounds **3g**) or two methoxy groups at positions 2 and 5 (compound **3j**) led to a decrease in antimicrobial activity.

Table 1. Antibacterial activity of tested compounds (**3a-j**) and antibiotics (MIC and MBC in μmol/ml x 10^{-2}).

Compounds		*B. cereus*	*M. flavus*	*S. aureus*	*L. monocy-togenes*	*E. coli*	*E. cloacae*	*P. aerugi-nosa*	*S. typhi-murium*
3a	**MIC**	3.06	4.50	6.25	1.625	6.25	3.06	6.25	6.25
	MBC	12.50	12.50	12.5	12.50	12.50	12.50	12.50	12.50
3b	**MIC**	1.625	6.25	3.06	6.25	6.25	6.25	6.25	3.06
	MBC	6.25	12.5	6.25	12.50	12.50	12.50	12.50	6.25
3c	**MIC**	1.625	3.06	1.625	6.25	6.25	6.25	1.625	1.625
	MBC	6.25	6.25	6.25	12.50	12.50	12.50	12.50	6.25
3d	**MIC**	3.06	3.06	0.90	6.25	6.25	3.06	1.53	1.53
	MBC	6.25	6.25	1.53	12.50	12.50	6.25	6.25	6.25
3e	**MIC**	43.00	21.50	43.00	43.00	21.50	43.00	43.00	21.50
	MBC	64.50	43.00	64.50	86.00	43.00	43.00	64.50	43.00
3f	**MIC**	30.00	3.06	30.00	1.53	1.53	3.06	0.90	0.90
	MBC	50.00	6.125	50.00	12.50	12.50	12.50	1.53	1.53
3g	**MIC**	10.50	21.00	42.00	42.00	21.00	2.00	42.00	21.00
	MBC	21.00	42.00	42.00	63.00	21.00	42.00	63.00	42.00
3h	**MIC**	1.625	6.125	3.06	6.125	8.50	3.06	3.06	3.06
	MBC	3.06	12.50	6.125	12.50	12.50	6.25	12.50	12.50
3i	**MIC**	1.63	6.25	3.06	6.25	3.06	3.06	3.06	3.06
	MBC	3.06	12.50	6.25	12.50	12.50	12.50	6.25	6.25
3j	**MIC**	21.90	21.90	43.80	43.80	21.90	21.90	43.80	21.90
	MBC	43.80	43.80	65.70	65.70	43.80	43.80	43.80	43.80
Strep.	**MIC**	4.30	8.60	17.20	25.80	17.20	4.30	27.20	17.20
	MBC	8.60	17.20	34.40	51.60	34.40	8.60	34.40	34.40
Amp.	**MIC**	24.80	24.80	24.80	37.20	37.20	24.79	74.40	24.80
	MBC	37.20	37.20	37.80	74.40	49.20	37.19	124.00	49.20

All the tested compounds showed very good antifungal activity against all tested fungi with MICs in a range of 0.021−87.60 μmol/ml x 10^{-2} and MFCs in a range of 0.045−109.50 μmol/ml x 10^{-2} (Table 2). The antifungal potential could be presented as follows: **3d> 3b> 3c> 3f> 3h> 3a> 3i> 3g>3e> 3j**.

The best fungistatic activity against all the tested fungi, except of *C. albicans,* was found for compound **3d**, which showed excellent activity with MIC values of only 0.021−0.042 μmol/ml x 10^{-2} and MFC values of 0.06 μmol/ml x 10^{-2}. Compound **3f** exhibited the best activity against *C. albicans* with MIC 0.08 μmol/ml x 10^{-2} and MFC 0.14 μmol/ml x 10^{-2}. The lowest fungistatic, as well as fungicidal, activity was achieved for compound **3j** (MIC 10.95−87.60 μmol/ml x 10^{-2} and MBC 21.9−109.50 μmol/ml x 10^{-2}).

Compounds **3a-c**, **3h** and **3i** also showed excellent antifungal activity with MICs and MFCs values slightly lower than compound **3d**. Lower, but still good, fungistatic and fungicidal activity, higher than that of ketoconazole and bifonazole, against all the tested fungi was shown by the following compounds: **3e**, **3g** and **3j**.

Considering all the tested fungi, for compounds **3a-d**, **3f**, **3h** and **3i**, *A. ochraceus* was proved to be the most sensitive, followed by *T. viride* and *P. funiculosum,* while *C. albicans* was the most resistant to the tested compounds, except the compound **3f**. In case of compounds **3e**, **3g** and **3j**, the most sensitive fungus appeared to be *T. viride* and *A. niger* the most resistant one.

All tested compounds showed excellent antifungal activity in comparison with commercial antimycotics. MICs for bifonazole were in a range of 32.0−64.0 μmol/ml x 10^{-2} and MFCs were in a range of 64.0−80.0 μmol/ml x 10^{-2}, while ketoconazole showed much lower activity with MICs from 38.0−475.0 μmol/ml x 10^{-2} and MFCs from 95.0−570.0 μmol/ml x 10^{-2}.

The relationship between structure and antifungal activity revealed that 2,3-diCl, 3-Cl and 4-Cl substitution of the phenyl ring (compounds **3d**, **3b** and **3c**, respectively) is favourable and in agreement with what observed for antibacterial potential. It was also found that presence of 3-F, 4-NO_2, 2-Cl and 4-OCH_3 groups lead to slightly less active compounds but still comparable with the most active ones. On the other hand, 4-Br, 2,6-diCl and 2,5-di-OCH_3 had negative effect to their antifungal activity.

According to the presented results it could be noticed that the antifungal potential of compounds **3a-d**, **3f**, **3h** and **3i** is higher than their antibacterial effect, while compounds **3e**, **3g** and **3j** exhibited decreased antibacterial as well as antifungal activity.

Table 2. Antifungal activity of tested compounds (**3a-j**) and fungicides (MIC and MFC in µmol/ml x 10^{-2}).

Compounds		*A. fumigatus*	*A. versicolor*	*A. ochraceus*	*A. niger*	*T. viride*	*P. funiculosum*	*P. ochrochloron*	*C. albicans*
3a	**MIC**	0.58	0.58	0.03	0.9	0.58	0.58	0.58	1.16
	MFC	1.16	1.16	0.07	1.16	1.16	1.16	1.16	2.32
3b	**MIC**	0.29	0.03	0.045	0.03	0.03	0.29	0.29	0.58
	MFC	0.58	0.07	0.07	0.07	0.045	0.58	0.58	1.16
3c	**MIC**	0.045	0.05	0.05	0.045	0.03	0.045	0.03	0.58
	MFC	0.07	0.07	0.07	0.07	0.05	0.07	0.07	1.16
3d	**MIC**	0.042	0.021	0.042	0.021	0.021	0.042	0.021	0.53
	MFC	0.06	0.06	0.06	0.06	0.06	0.06	0.06	1.075
3e	**MIC**	21.48	21.48	10.74	21.48	21.48	21.48	21.48	42.96
	MFC	53.71	32.22	53.71	32.22	21.48	53.71	32.22	64.45
3f	**MIC**	0.08	0.04	0.04	0.08	0.12	0.08	0.04	0.08
	MFC	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
3g	**MIC**	21.02	10.51	10.51	21.02	10.51	21.02	21.02	52.58
	MFC	21.02	10.51	42.04	31.54	10.51	52.58	21.02	73.60
3h	**MIC**	1.13	0.56	0.14	0.35	0.14	0.07	0.14	1.13
	MFC	4.52	1.13	0.28	0.56	0.28	0.14	0.28	2.26
3i	**MIC**	1.17	0.29	0.14	0.29	0.14	0.29	0.09	1.17
	MFC	2.34	0.58	0.29	0.58	0.29	0.58	0.14	2.34
3j	**MIC**	21.90	21.90	10.95	21.90	21.90	21.90	21.90	87.60
	MFC	32.85	32.85	32.85	87.60	21.90	43.80	43.80	109.50
Ket.	**MIC**	38.00	38.00	38.00	38.00	475.00	38.00	380.00	38.00
	MFC	95.00	95.00	95.00	95.00	570.00	95.00	380.00	95.00
Bif.	**MIC**	48.00	32.00	48.00	48.00	64.00	64.00	48.00	32.00
	MFC	64.00	64.00	80.00	64.00	80.00	80.00	64.00	48.00

2.2.2 HIV-RT inhibitory activity evaluation

Based on our previous results that compounds **3e** and **3g** had shown some inhibitory activity against HIV-1 reverse transcriptase,[27] compounds **3a-d, 3f** and **3h-j** were evaluated for HIV-1 reverse transcriptase inhibitory activity. The obtained data is presented in Table 3.

Table 3. HIV-RT inhibitory activity of tested compounds **(3a-j)**.

Compounds	[I] (µM)	Residual RT Activity (%)
3a	4.00	99.12
3b	4.00	99.35
3c	4.00	99.05
3d	4.00	100.20
3e	4.00	52.52
3f	4.00	100.03
3g	4.00	59.85
3h	4.00	99.27
3i	4.00	100.17
3j	4.00	80.65
DMSO (4%)		100
Efavirenz	0.10	3.10

All compounds were assayed at the final concentrations of 4µM. Only compounds **3e** and **3g** showed moderate inhibitory activity and ID_{50} values (µM) were determined and reported in our previous publication.[27] However, none of the other compounds showed significant inhibitory activity at these concentrations.

2.3 Docking studies on MurB protein of *S.aureus*

In order to explore the possible binding mode at MurB protein of *S.aureus* (PDB ID: 1HSK), we performed docking studies for the most active (**3d**) (MIC = 0.9 µmol/ml x 10^{-2}) and one of the least active (**3e**) (MIC = 43 µmol/ml x 10^{-2}) compounds using the GLIDE module implemented in Schrödinger software package (Figure 1). The important interactions of the most active compound **3d** (R-isomer) included hydrogen bond contacts with the backbone nitrogen of GLY249 and side chain nitrogen of ARG242. Apart from the hydrogen bond contacts, compound **3d** was involved in the "edge on" Cl-π interaction with Phe274 at the active site. The 3-Cl substituent attached to the phenyl ring of compound **3d** had a distance (r=3.65 Å) from centroid of PHE274 and a distance (r=3.13 Å) from one of the ring carbon atoms as shown in Fig.1C. The difference between |r-r'| was 0.52Å (value above 0.3Å) that indicated an "edge on" Cl-π interaction with Phe274.[50] The phenyl ring in 2,3-dichlorophenyl moiety was in hydrophobic contact with VAL239 and GLY273, with additional face to face π-π interaction with PHE274. The Cl-π interaction

was the determining factor governing the activity of the compounds in the halogen series, as observed in the least active compound **3e** that has 2,6- dichloro moiety far away from Phe274 and thus was unable to show Cl-π interaction. The thiazolidine-4-one is a potential surrogate of the di-phosphate moiety, present in UDP-N-acetylenol-pyruvylglucosamine.[51] The keto-group of the thiazolidine moiety formed a hydrogen bond with GLY249 and the electronegative sulphur atom forms a hydrogen bond with the side chain of ARG242 that has been postulated as an important amino acid involved in UDP-N-acetylenol-pyruvylglucosamine binding.[52] The thiazole moiety, attached to the thiazolidine-4-one, formed π-π interaction with another important residue HIS271.[52] The adamantane moiety resided at the shallow pocket formed by the GLY249, GLN 253, and ALA272. Thus, the Cl-π interaction appeared to be the main determinant for enhanced activity.

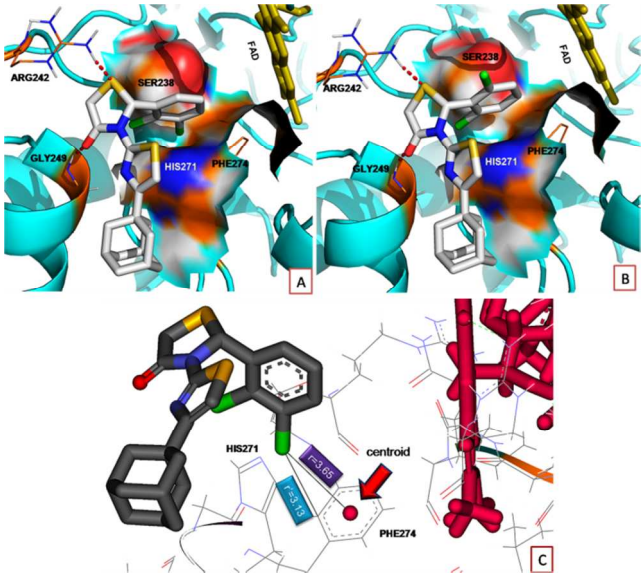


Figure 1. (A) The docked conformation of the most active compound (**3d**) and **(B)** one of the least active compounds (**3e**) at the active site of the MurB protein of *S.aureus*. **(C)** The Cl-π interaction of 3-Cl substituent in compound **3d**.

3. Experimental

All commercial reagents and solvents were used without further purification. Reactions were monitored by TLC on silica gel with detection by UV light (254 nm) and iodine. TLC analysis was performed using Polygram® precoated silica gel TLC sheets SIL G/UV_{254}. Melting points of the compounds were determined using a MELTEMP II capillary apparatus (LAB Devices, Holliston, MA, USA) and are uncorrected. All microwave-assisted reactions were carried out in a dedicated CEM-Discover monomode microwave apparatus, operating at a frequency of 2.45GHz with continuous irradiation power from 0 to 300W with utilization of the standard absorbance level of 300W maximum power. Elemental analyses were performed on a Perkin–Elmer 2400 CHN elemental analyzer (The Perkin-Elmer Corporation Ltd., Lane Beaconsfield, Bucks, UK) and all compounds synthesized and were within a 0.4% of theoretical values. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance III nanobay ultra shield 400 spectrometer or Brucker AC 300 spectrometer. The chemical shift (δ) values are expressed in parts per million (ppm) and coupling constants are in Hertz (Hz). DMSO-d_6 was used as the standard NMR solvent. Legend: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet.

ESI-MS spectra were obtained with an Esquire 3000 plus ion trap mass spectrometer from Bruker Daltonics, using the direct infusion mode. UPLC (Ultra Performance Liquid Chromatography): Waters acquity H-class UPLC system coupled to a waters TQD ESI mass spectrometer and waters TUV detector. A waters acquity UPLC BEH C18 1.7µm 2.1-50 mm column was used. Solvent A: water with 0.1% formic acid, solvent B: acetonitrile with 0.1% formic acid. Method: 0.15min 95% A, 5% B, then in 1.85 min from 95% A, 5% B to 95% B, 5% A, then 0.25min (0.350ml/min), 95% B, 5% A. The wavelength for UV detection was 254nm. The quasi-molecular ions $[M+H]^+$ were detected.

3.1 Procedure for the synthesis of 4-[(3r,5r,7r-) adamantan-1-yl] thiazol-2-amine (2)

A suspension of thiourea (0.59g, 7.75 mmol, 2eq.) in isopropanol (39ml) was added to a solution of 1-adamantyl bromomethyl ketone (1g, 3.89 mmol, 1eq) in isopropanol (19.3ml). The mixture was stirred at room temperature for 30 minutes. Subsequently, the reaction mixture was poured into an aqueous solution of sodium carbonate (5% w/v) and the formed precipitate was filtered and recrystallized from ethyl acetate to deliver the target compound.

3.2. General procedure for the synthesis of final compounds (3a-j)

A mixture of 4-(adamantan-1-yl)thiazol-2-amine (1.0 mmol), the appropriate substituted benzaldehyde (1.5 mmol) and mercaptoacetic acid (5 mmol) was placed in a 10 mL reaction vial containing absolute ethanol (~3 mL) and a stirring bar. The vial was sealed tightly with a teflon septum, placed into the microwave cavity and irradiated at 110–130 °C using 100−150 W as maximum power for 20–60 min. Then, the reaction mixture was rapidly cooled with gas jet cooling to ambient temperature. The corresponding final compound precipitated after cooling and was collected by filtration. The precipitate was taken up with ethyl acetate and the organic layer was washed with aqueous citric acid (5% w/v), water and aqueous sodium hydrogen carbonate (5% w/v). The organic layer was dried over sodium sulfate and evaporated under reduced pressure to give the pure product.

3.2.1. 3-(4-(Adamantan-1-yl)thiazol-2-yl)-2-(2-chlorophenyl)thiazolidin-4-one (3a)

Yield: 44%, mp: 112−115 °C, R_f = 0.80 (petroleum ether/ ethylacetate: 8/2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.64–7.58 (m, 1H, Ph), 7.47–7.38 (m, 1H, Ph), 7.38–7.28 (m, 2H, Ph), 6.77 (s, 1H, thiazole, 5-H), 6.63 (d, J = 1.0 Hz, 1H, thiazolidinone, 2-H), 4.39 (dd, J = 16.4, 1.3 Hz, 1H, thiazolidinone, 5-H_AH_B), 4.02 (d, J = 16.4 Hz, 1H, thiazolidinone, 5-H_AH_B), 1.96 (s, 3H, Ad), 1.74-1.60 (m, 12H, Ad). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.1, 160.2, 154.8, 144.0, 132.6, 130.3, 127.9, 127.7, 125.0, 105.6, 62.5, 41.3, 36.3, 35.8, 32.4, 27.9. MS (ESI), *m/z*: 431.1, 433.1 (3:1) $[M+H]^+$. Anal. Calcd for $C_{22}H_{23}ClN_2OS_2$ (MW 431): C, 61.31; H, 5.38;N, 6.50%. Found: C, 61.48; H, 5.30; N, 6.47%.

3.2.2. 3-(4-(Adamantan-1-yl)thiazol-2-yl)-2-(3-chlorophenyl)thiazolidin-4-one (3b)

Yield: 20%, mp: 90−93 °C, R_f = 0.84 (petroleum ether/ethylacetate: 8/2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (s, 1H, Ph), 7.44 (s, 1H, Ph), 7.39–7.26 (m, 2H, Ph), 6.76 (s, 1H, thiazole, 5-H), 6.63 (s, 1H, thiazolidinone, 2-H), 4.39 (d, J = 16.3 Hz, 1H, thiazolidinone, 5-H_AH$_B$), 4.02 (d, J = 16.5 Hz, 1H, thiazolidinone, 5-H$_A$$H_B$), 1.96 (s, 3H, Ad), 1.76–1.59 (m, 12H, Ad). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.1, 160.2, 154.8, 144.0, 132.6, 130.3, 127.9, 127.7, 125.0, 105.6, 62.5, 41.3, 36.2, 35.8, 32.4, 27.9. MS (ESI), m/z: 431.2, 433.1 (3.1) [M+H]$^+$. Anal. Calcd for $C_{22}H_{23}ClN_2OS_2$ (MW 431): C, 61.31; H, 5.38; N, 6.50%. Found: C, 61.48; H, 5.25; N, 6.46%.

3.2.3. 3-(4-(Adamantan-1-yl)thiazol-2-yl)-2-(4-chlorophenyl)thiazolidin-4-one (3c)

Yield: 36%, mp: 183−185 °C, R_f = 0.86 (petroleum ether/ethylacetate: 8/2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.49–7.47 (m, 1H, Ph), 7.47–7.45 (m, 1H, Ph), 7.40–7.37 (m, 1H, Ph), 7.37–7.35 (m, 1H, Ph), 6.77 (s, 1H, thiazole, 5-H), 6.66 (d, J = 0.9 Hz, 1H, thiazolidinone, 2-H), 4.31 (dd, J = 16.5, 1.3 Hz, 1H, thiazolidinone, 5-H_AH$_B$), 4.01 (d, J = 16.5 Hz, 1H, thiazolidinone, 5-H$_A$$H_B$), 1.95 (s, 3H, Ad), 1.74−1.58 (m, 12H, Ad). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.2, 160.3, 154.9, 140.6, 132.4, 128.5, 128.2, 105.7, 62.5, 41.2, 36.3, 35.7, 32.3, 27.8. MS (ESI), m/z: 431.1, 433.0 (3:1) [M+H]$^+$. Anal. Calcd for $C_{22}H_{23}ClN_2OS_2$ (MW 431): C, 61.31; H, 5.38;N, 6.50%. Found: C, 61.50; H, 5.20; N, 6.57%.

3.2.4. 3-(4-(Adamantan-1-yl)thiazol-2-yl)-2-(2,3-dichlorophenyl)thiazolidin-4-one (3d)

Yield: 60%, mp: 154−156 °C, R_f = 0.80 (petroleum ether/ethylacetate: 8/2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56 (dd, J = 7.4, 2.0 Hz, 1H, Ph), 7.36–7.24 (m, 2H, Ph), 6.93 (d, J = 1.2 Hz, 1H, thiazole, 5-H), 6.75 (s, 1H, thiazolidinone, 2-H), 4.26 (dd, J = 16.2, 1.4 Hz, 1H, thiazolidinone, 5-H_AH$_B$), 4.08 (d, J = 16.2 Hz, 1H, thiazolidinone, 5-H$_A$$H_B$), 1.91 (s, 3H, Ad), 1.78–1.46 (m, 12H, Ad). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.1, 160.1, 154.8, 141.1, 131.9, 129.7, 128.6, 127.4, 125.6, 105.6, 60.4, 41.0, 36.2, 35.7, 32.4, 27.8. MS (ESI), m/z: 465.1, 467.1, 469.1 (9:6:1) [M+H]$^+$. Anal. Calcd for $C_{22}H_{22}Cl_2N_2OS_2$ (MW 465): C, 56.77; H, 4.76; N, 6.02%. Found: C, 56.71; H, 4.79; N, 6.06%.

3.2.5. 3-(4-(Adamantan-1-yl)thiazol-2-yl)-2-(3-fluorophenyl)thiazolidin-4-one (3f)

Yield: 39%, mp: 96−99 °C, R_f = 0.81 (petroleum ether/ethylacetate: 8/2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.41–7.24 (m, 3H, Ph), 7.14–7.04 (m, 1H, Ph), 6.77 (s, 1H, thiazole, 5-H), 6.66 (s, 1H, thiazolidinone, 2-H), 4.36 (dd, J = 16.4, 1.2 Hz, 1H, thiazolidinone, 5-H_AH$_B$), 4.01 (d, J = 16.4 Hz, 1H, thiazolidinone, 5-H$_A$$H_B$), 1.94 (s, 3H, Ad), 1.73−1.58 (m, 12H, Ad). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.2, 161.9 (d, $^1J_{C\text{-}F}$ = 243.8 Hz), 160.2, 154.9, 144.5 (d, $^3J_{C\text{-}F}$ = 7.2 Hz), 130.3 (d, $^3J_{C\text{-}F}$ = 8.3 Hz), 122.4 (d, $^4J_{C\text{-}F}$ = 2.7 Hz), 114.8 (d, $^2J_{C\text{-}F}$ = 21.1 Hz), 113.8 (d, $^2J_{C\text{-}F}$ = 22.5 Hz), 105.6, 62.5 (d, $^3J_{C\text{-}F}$ = 1.8 Hz), 41.2, 36.2, 35.7, 32.2, 27.8. MS (ESI), m/z: 415.2 [M+H]$^+$. Anal. Calcd for $C_{22}H_{23}FN_2OS_2$ (MW 414): C, 63.74; H, 5.59; N, 6.76%. Found: C, 63.70; H, 5.61; N, 6.73%.

3.2.6. 3-(4-(Adamantan-1-yl)thiazol-2-yl)-2-(4-nitrophenyl)thiazolidin-4-one (3h)

Yield: 44%, mp: 190−193 °C, R_f = 0.72 (petroleum ether/ethylacetate: 8/2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, J = 8.7 Hz, 2H, Ph), 7.72 (d, J = 8.7 Hz, 2H, Ph), 6.79 (d, J = 5.4 Hz, 2H, thiazole, 5-H and thiazolidinone, 2-H), 4.32 (d, J = 16.5 Hz, 1H, thiazolidinone, 5-H_AH$_B$), 4.05 (d, J = 16.4 Hz, 1H, thiazolidinone, 5-H$_A$$H_B$), 1.91 (s, 3H, Ad), 1.71–1.46 (m, 12H, Ad). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.2, 160.2, 154.9, 149.2, 146.9, 127.7, 123.6, 105.8, 62.1, 41.2, 36.2, 35.7, 32.3, 27.8. MS (ESI), m/z: 442.2 [M+H]$^+$. Anal. Calcd for $C_{22}H_{23}N_3O_3S_2$ (MW 441): C, 59.84; H, 5.25; N, 10.87%. Found: C, 59.80; H, 5.26; N, 10.84%.

3.2.7. 3-(4-(Adamantan-1-yl)thiazol-2-yl)-2-(4-methoxyphenyl)thiazolidin-4-one (3i)

Yield: 24%, mp: 114−116 °C, R_f = 0.67 (petroleum ether/ethylacetate: 8/2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.39 (d, J = 8.6 Hz, 2H, Ph), 6.86 (d, J = 8.6 Hz, 2H, Ph), 6.76 (s, 1H, thiazole, 5-H), 6.62 (s, 1H, thiazolidinone, 2-H), 4.31 (d, J = 16.5 Hz, 1H, thiazolidinone, 5-H_AH$_B$), 3.99 (d, J = 16.5 Hz, 1H, thiazolidinone, 5-H$_A$$H_B$), 3.71 (s, 3H, CH_3O), 1.96 (s, 3H, Ad), 1.76–1.60 (m, 12H, Ad). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.2, 160.3, 159.0, 154.9, 133.3, 128.2, 113.6, 105.6, 62.9, 55.2, 41.3, 36.3, 35.8, 32.4, 27.9. MS (ESI), m/z: 427.2 [M+H]$^+$. Anal. Calcd for $C_{23}H_{26}N_2O_2S_2$ (MW 426): C, 64.76; H, 6.14; N, 6.57%. Found: C, 64.71;, H, 6.20; N, 6.60%.

3.2.8. 3-[4-(2-Adamantyl)-1,3-thiazol-2-yl]-2-(2,5-dimethoxyphenyl)-1,3-thiazolidin-4-one (3j)

Yield: 31%, mp: 161−162 °C, R_f = 0.62 (petroleum ether/ethylacetate: 8/2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.94 (d, J = 9.0 Hz, 1H, Ph), 6.83–6.77 (m, 2H, Ph), 6.74 (s, 1H, thiazole, 5-H), 6.72 (d, J = 1.2 Hz, 1H, thiazolidinone, 2-H), 4.15 (dd, J = 16.2, 1.4 Hz, 1H, thiazolidinone, 5-H_AH$_B$), 3.98 (d, J = 16.2 Hz, 1H, thiazolidinone, 5-H$_A$$H_B$), 3.77 (s, 3H, CH_3O), 3.66 (s, 3H, CH_3O), 1.94 (s, 3H, Ad), 1.73–1.57 (m, 12H, Ad). ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.6, 160.2, 154.9, 152.7, 150.6, 129.5, 113.9, 113.7, 112.6, 105.4, 59.7, 56.3, 55.3, 41.2, 36.3, 35.7, 33.0, 27.8. MS (ESI), m/z: 457.2 [M+H]$^+$. Anal. Calcd for $C_{22}H_{22}Cl_2N_2OS_2$ (MW 456): C, 63.13; H, 6.18; N, 6.13%. Found: C, 63.18; H, 6.15; N, 6,16%.

3.3. Biological evaluation

3.3.1. Antibacterial activity

The following Gram positive bacteria: *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), *Staphylococcus aureus* (ATCC 6538) and *Listeria monocytogenes* (NCTC 7973) and Gram negative bacteria: *Escherichia coli* (ATCC 35210), *Enterobacter cloacae* (human isolate), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhimurium* (ATCC 13311) were used. The microorganisms were obtained from the Mycological laboratory, Department of Plant Physiology, Institute for biological research "Sinisa Stanković", University of Belgrade, Serbia.

The minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined by the microdilution method.[53,54] Briefly, fresh overnight culture of bacteria was adjusted by the spectrophotometer to a concentration of 1×10^5 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 in

sterile water and added in broth Triptic Soy broth (TSB) medium (100 µL) with bacterial inoculum (1.0×10^4 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 37 ^{0}C. The MICs of the samples were 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.

MBCs were 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. Standard drugs, namely streptomycin and ampicillin were used as positive controls. 5% of DMSO in sterile water was used as negative control. All experiments were performed in duplicate and repeated three times.

3.3.2. Antifungal activity

The used fungi: *Aspergillus fumigatus* (ATCC 1022), *Aspergillus versicolor* (ATCC 11730), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger* (ATCC 6275), *Trichoderma viride* (IAM 5061), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112) and *Candida albicans* (ATCC 10231) were obtained from Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia.

The micromycetes were maintained on malt agar and the cultures were stored at 4 °C and sub-cultured once a month.[55] The antifungal assay was carried out by modified microdilution technique.[56] 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 inoculum were cultured on solid malt agar to verify the absence of contamination and to check the validity of the inoculum.

MIC determinations were performed by a serial dilution technique using 96-well microtiter plates. The examined compounds were diluted in 5% of DMSO in sterile water (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.

3.3.3. In vitro HIV-RT kit assay

RNA-dependent DNA polymerase activity of HIV-1 reverse transcriptase (RT) was assayed in a reaction buffer containing 50mM Tris-HCl (pH 8.0), 1mM dithiothreitol (DTT), 0.2 mg/ml bovine serum albumine (BSA) and 2% glycerol. Thus, 2-4 nM RT were incubated at 37 °C with 10 mM $MgCl_2$, 0.5 µg of poly(rA):oligo(dT)$_{10:1}$ (0.3 µM 3'-OH ends), 10 µM radioactive 2'deoxy-thymidine 5'-triphosphate (^{3}[H]dTTP) (1 Ci/mmol), for 15 min. Then, 20 µl aliquots were spiked on glass fiber filters GF/C (Whatman Int. Ltd, Maidstone, England) and, immediately, immersed in 5% ice-cold trichloroacetic acid (TCA) (AppliChem GmbH, Darmstadt). Filters were washed three times with 5% TCA and once with ethanol for 5 minutes, then dried and, finally, added with EcoLume® Scintillation cocktail (ICN, Research Products Division, Costa Mesa, CA USA) to detect the acid-precipitable radioactivity by scintillation counting. Incorporation of radioactive dTTP into poly(rA):oligo (dT) was tested in the absence or presence of the tested compounds (4µM).

3.4. Docking studies

3.4.1. Ligand and protein preparation

The protein preparation was performed using Protein Preparation Wizard[57] implemented in Schrödinger 9 suite.[58] The 3D structures of the ligands were sketched in the maestro workspace using the drawing tools in maestro window. The 3D structures were geometry optimized by clean up geometry and subsequent ligand preparation was performed utilizing the Ligprep module[59] in Maestro (version 9.0).

3.4.2. Molecular Docking and Scoring

The molecular docking of the compounds was executed using the GLIDE[60] module implemented in Schrödinger 9 suite.[58] The receptor grid was generated using the centroid of the residues ARG188, SER238, ARG242, HIS271 and GLU308 of PDB 1HSK as they are proposed to be important for binding of enolpyruvyl-UDP-N-acetylglucosamine (EP-UDPGlcNAc) with *S. aureus* MurB.[52] The distance criteria for Cl-π interactions was visualized and measured using the Discovery Studio 2.0 (DS 2.0)[61] software.

Conclusions

All tested compounds **3a-j** exhibited a remarkable growth inhibition against a wide spectrum of Gram-positive bacteria, Gram-negative bacteria and fungi. It is noteworthy that all the compounds exhibited better or comparable activity with the commercial antimicrobial/mycotic agents used as reference drugs (ampicillin, streptomycin, bifonazole and ketoconazole). Compound **3d** displayed the best antimicrobial profile with MICs in a range of 0.9-6.25 µmol/ml x 10^{-2} and MBCs in a range of 1.53-12.5 µmol/ml x 10^{-2}, as well as, the best antifungal profile with MICs from 0.021-0.53 µmol/ml x 10^{-2} and MFCs from 0.06-1.075 µmol/ml x 10^{-2}.

The most sensitive bacterial species to the tested compounds were *S. typhimurium* and *M. flavus*; while *L. monocytogenes* was the most resistant one. Considering all the tested fungi, *A. ochraceus* was proved to be the most sensitive, while *C. albicans* was the most resistant**.**

Moreover, the title compounds were screened for HIV-1 RT inhibitory activity, although none of them showed significant activity. Furthermore, docking studies for compounds **3d** and **3e** were performed in order to explore their possible binding mode at MurB protein of *S.aureus.*

The promising 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.

Notes and references

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