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ORIGINAL ARTICLE

Design, synthesis and biological evaluation of new substituted 5-benzylideno-2-adamantylthiazol[3,2-b][1,2,4]triazol-6(5*H*)ones. Pharmacophore models for antifungal activity

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Abstract As a part of our ongoing studies in developing new derivatives as antimicrobial agents we describe the synthesis of novel substituted 5-benzylideno-2-adamantylthiazol[3,2-b][1,2,4]triazol-6 (5H)ones.The twenty-five newly synthesized compounds were tested for their antimicrobial and antifungal activity. All compounds have shown antibacterial properties with compounds **1–9** showing the lowest activity, followed by compounds **10–14** while compounds **15–25** the highest antibacterial activity. Specific compounds appeared to be more active than ampicillin in most studied strains and in some cases more active than streptomycin. Antifungal activity in most cases also was better than that of reference drugs ketoconazole and bifonazole. Elucidating the relation of

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molecular properties to antimicrobial activity as well as generation of pharmacophore model for antifungal activity of two fungal species *Aspergillus fumigatus* and *Candida albicans* were performed.

1. Introduction

During the past 50 years the significant efforts in the diagnosis and treatment of microbial diseases (Fernandes, 2006) led to impressive gains in the treatment of microbial diseases introducing a range of therapeutic strategies in clinical practice. However, in spite of a large number of antibiotics and chemotherapeutics available for medical use there is still an urgent medical need for new classes of antibacterial agents, due to the emergence of old and new antibiotics' resistance created in the last decades. A potential approach that addresses this issue of resistance is the design of novel agents with different mode of action in order to avoid the occurrence of cross resistance with the present therapeutics.

Five member heterocycles with two of three heteroatoms, such as imidazoles, thiazoles, triazoles and others are key structural units in many pharmaceutical preparations.

Specifically, 1,2,4-triazoles and their heterocyclic derivatives represent an interesting class of compounds possessing a wide spectrum of biological activities. A large number of 1,2,4,-triazole derivatives containing ring systems exhibit antibacterial (Gabriela et al., 2010; Upmanyu et al., 2011; Prasad et al., 2012; Taj et al., 2013; Kumar et al., 2014; Gupta et al., 2015), antifungal (Sangshetti et al., 2009; Zoumpoulakis et al., 2012; Barbuceanu et al., 2009, 2012; Sahu et al., 2014; Gupta et al., 2015) antitubercular (Gill et al., 2008; Kumar et al., 2010; Cristophe et al., 2011; Godhani et al., 2015), analgesic (Amir et al., 2008;Tozkoparan et al., 2012; Khanage et al., 2013; Sarigol et al., 2015), anti-inflammatory (Pattan et al., 2012; Ayse et al., 2012; Ashour et al., 2013; Sarigol et al., 2015), anticancer (Romagnoli et al., 2010; Wang et al., 2011; Bai et al., 2012), anticonvulsant (Siddiqui et al., 2010; Dayanand et al., 2011; Botros et al., 2013; Plech et al., 2013; Kamboj et al., 2015), antiviral (Abdel-Aal et al., 2008; Jordao et al., 2009; El-Etrawy et al., 2010), antimalarial (Mishra et al., 2008; Gujjar et al., 2009) central nervous system (Kamboj et al., 2015) and other activities (Puthiyapurayil et al., 2012; Iqbal et al., 2012).

Additionally, the thiazolyl group bears great importance in biological systems. In this context, thiazole derivatives find a variety of applications such as bacteriostatics (Abdel-Wahab et al., 2009; Dawane et al., 2010; Kouatly et al., 2010; Zablotskaya et al., 2013; Haroun et al., 2016), antibiotics (Mostafa and Abd El-Salam, 2013), antifungal (Bharti et al., 2010), CNS regulants of high selling diuretics (Sucman et al., 2011), local anaesthetics (Geronikaki et al., 2009), anti-inflammatory (Lagunin et al., 2008; Kouatly et al., 2009; Pattan et al., 2009; Apostolidis et al., 2013), analgesic and antipyretics (Pattan et al., 2009; Saravanan et al., 2011), HIV infections (Pitta et al., 2010, 2013), antiallergic (Hargrave et al., 1983), antihypertensives (Abdel-Wagab et al., 2008), against schizophrenia (Gupta, 2013), antidiabetic (Lino et al., 2009), anthelminthic (Amnerkar and Bhusari, 2011), anticancer (Luzina and Popov, 2009; Liu et al., 2009) and antioxidant (Gouda et al., Geronikaki et al., 2013). Furthermore, the thiazole ring is also found in many potent biologically active molecules. In particular, Thiabendazole and 2-(p-chlorophenyl) thiazole-4-acetic acid are widely used as anti-inflammatory drugs (van Arman and Campbell, 1975). Meloxicam is a new NSAID with a thiazolyl group in its structure (Kumar and Mishra, 2006). Some other thiazole derivatives are antiulcer (Nizatidine), antiretroviral (Ritonavir) (De Souza and De Almeida, 2003) agents, while others (Van Arman and Campbell, 1975; Ramachandran et al., 2011; Vicini et al., 2008) as well as Niridazole (Kilpatrick et al., 1982) have been found to exhibit antimicrobial antifungal/antihelminthic activities.

Another interesting core in medicinal chemistry responsible for numerous pharmacological properties and biological activities is the thiazolidinone (Knutsen et al., 2007; Apostolidis et al., 2013). Many publications refer to antifungal activity of different thiazole and thiazolidinone derivatives (Amnerkar and Bhusari, 2011; Apostolidis et al., 2013; Marques et al. 2014; Gupta et al., 2016; Haroun et al., 2016).

In view of these facts the thiazolo[3,2-b]1,2,4-triazoles are compounds with broad spectrum of biological activities, such as antimicrobial (Barbuceanou et al., 2009; Karthikeyan, 2009; Gupta et al., 2015), anticancer (Lesyk et al., 2007; Kaminskyy et al., 2011), anti-inflammatory (Tozkoparan et al., 2000; Doğdaş et al., 2007; Apostolidis et al., 2013) and analgesic (Assarzadeh, 2014) as well as antihypertensive (Bhandari et al., 2009) and anti-diabetic (Calderone et al., 2009).

Moreover, adamantane derivatives have been documented for their antiviral activity against influenza A (McSharry et al., 2007; Galvão et al., 2014) and HIV viruses (Balzarini et al., 2009; Pitta et al., 2010). Several adamantane derivatives were also associated with central nervous system (Suh et al., 2005), antimicrobial (Kadi et al., 2007) and anti-inflammatory activities (Kadi et al., 2007; Tozkoparan et al., 2012; Karthikeyan, 2008; Kouatly et al., 2009).

These findings focused particular interest on the incorporation of thiazolo[3,2-b]1,2,4-triazole with adamantine ring in one frame in order to obtain compounds with improved/higher antibacterial and antifungal activities.

To this extend, twenty-five new 5-arylidene **-2**-adamatylthiazol[3,3-b]triazol-6(5H)-ones (Scheme 1,1–25) were synthesized and evaluated for their *in vitro* antimicrobial properties against Gram positive, Gram negative bacteria and fungi strains. To a step further, multivariate data analysis highlighted the relation between molecular properties to antimicrobial and antifungal activities of the synthesized compounds.

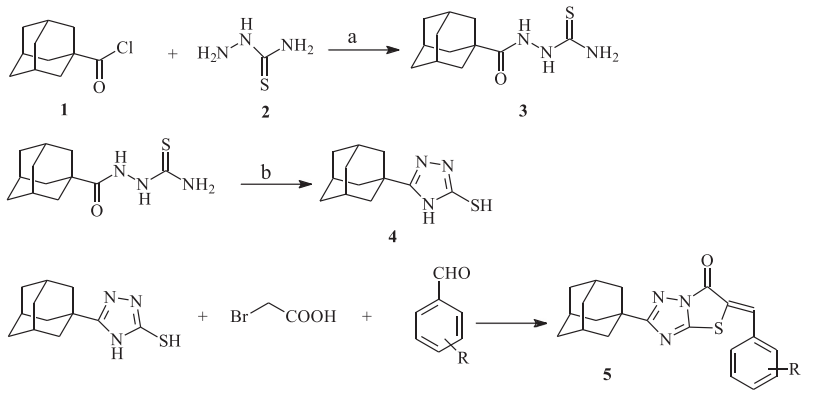
2. Results and discussion

2.1. Chemistry

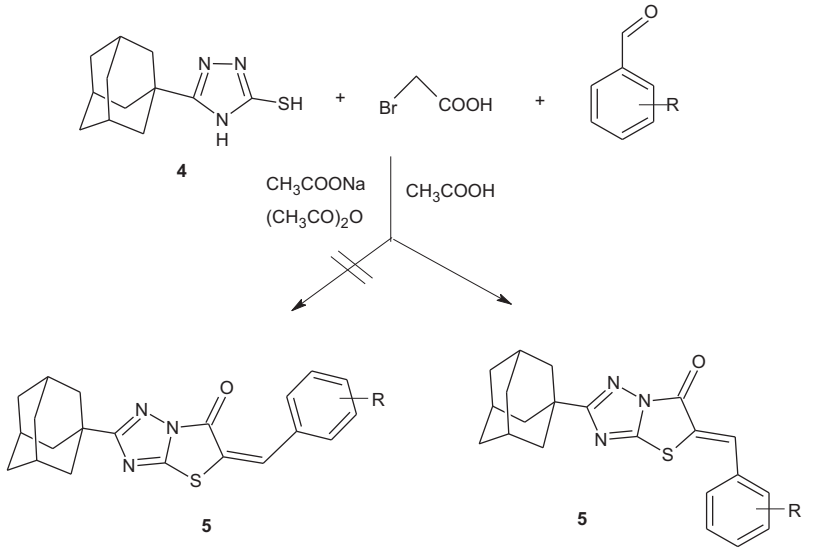
The synthesis of title compounds was performed by a multistep reaction as shown in Scheme 1. Adamantane thiosemicarbizide (3) was synthesized using a procedure reported earlier starting from adamantine-1-carbonyl chloride (1) upon reaction with thiosemicarbazide (2), followed by cyclization in alkaline solution under reflux to 5-adamantyl-4H-1,2,4-trizol-3-thiole (3). The third step includes the one pot condensation of 5-adamantyl-4H-1,2,4-triazol-3-thiole (4) with bromoacetic acid and appropriate substituted benzaldehydes in the presence of sodium acetate and acetic anhydride (Karthikeyan et al., 2008). Reactions proceed smoothly with good yields (55–88%).

All new structures of compounds **1–25** were characterized by IR, ^{1}H NMR and elemental analysis. IR spectra showed absorptions at 1724–1747 cm^{-1} (C═O) and at 1578–1654 cm^{-1} (C═N). In the ^{1}H NMR spectra the title compounds showed peaks in the region of 1.75–2.36 ppm (adamantine), 7.12–7.80 ppm (Ar─H) and 8.11–8.48 ppm (CH═).

During the reaction of 5-adamantyl-4*H*-1,2,4-trizol-3-thiole with different dielectrophiles the formation of two cyclic



Scheme 1 Synthesis of 5-benzylideno-2-adamantyltheiazol[3,2-b][1,2,4]triazol-6(5*H*)ones.



Scheme 2 Formation of thiazolo[3,2-b]-1,2,4-triazole.

isomers, (a) thiazolo[3,2-b]-1,2,4-triazole and (b) thiazolo[3,2-c]-1,2,4-triazole is possible (Karthikeyan, 2008) (Scheme 2).

Compounds **1–25** exist as potential E and Z geometrical isomers; the Z conformation of the 5 exocyclic C═C double bond was assigned on the basis of literature data for analogues structures (Ottanà et al., 2005) as well as on experimental data (^{1}H NMR) by comparing the resonance region of the hydrogens of the 5-adamantyl-4*H*-1,2,4-triazole-3-thiole to the corresponding ones of the final compounds (Scheme 2). The adamantane protons of thiol group resonated at 1.70–2.51 ppm, while the adamantane protons of the title compounds resonated at 1.75–2.36 ppm. It is obvious that both the title and intermediate compounds showed peaks for adamantane hydrogens in the same area, confirming the formation of the Z isomer thiazolo[3,2-b]-1,2,4-triazole. On the contrary, in case of the formation of E isomer, the aromatic ring protons will appear at higher chemical shift values, deshielded by the adjacent C═O.

2.2. Antimicrobial activity

The results of antibacterial activity of the compounds **1–25** are presented in Table 1. Almost all the tested compounds showed antibacterial activity but on different level. Compounds **15–25** showed higher antibacterial potential than others with MIC ranged between 4.5–26.4 $*$ 10^{-2} μmol/mL and MBC 9.0–42.2 $*$ 10^{-2} μmol/mL. The majority of the compounds were inactive against *Micrococcus flavus*. Compound **15**, did not show activity at tested concentration against *M. flavus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *E. faecalis,* **19** and **25** on *P. aerugunisa* and *E. faecalis*, **22** on *Bacillus cereus, Escherichia coli* and *E. faecalis,* while **21** on *E. coli* and *E. faecalis*. The antibacterial potential of tested compounds could be presented as follows: **18 > 24 > 23 > 17 > 16 > 19 > 20 > 25 > 21 > 22 > 15**. It can be seen that compound **18** showed the best antibacterial activity with MIC in the interval 4.90–9.80 $*$ 10^{-2} μmol/mL and MBC 9.80–36.6 $*$ 10^{-2} μmol/mL. This compound is followed by **24** with MIC at 4.5–13.6 $*$ 10^{-2} μmol/mL and MBC between 9.0–27.1 $*$ 10^{-2} μmol/mL. The lowest antibacterial activity among all tested compounds was obtained for compound **15** with inhibitory activity at 5.3–26.4 $*$ 10^{-2} μmol/mL and bactericidal effect at .10.5- > 42.2 $*$ 10^{-2} μmol/mL. Ampicillin showed inhibitory effect at 24.8–74.4 $*$ 10^{-2} μmol/mL and bactericidal at 37.2–124.0 $*$ 10^{-2} μmol/mL, while Streptomycin showed MIC in range of

Table 1 Antibacterial activity of XK compounds (MIC and MBC, μmo/ml × 10^{-2}).

Compounds		*S.a.*	*B.c.*	*M. f*	*L. m.*	*Ps. aer.*	*S. typhi*	*E. coli*	*En.faec*	*En.cl*
1	**MIC**	225 ± 1.7^{g}	550 ± 1.7^{h}	–	110 ± 3.3^{j}	220 ± 1.7^{d}	220 ± 0.7^{c}	–	330 ± 1.0^{f}	–
	MBC	550 ± 1.7^{h}	550 ± 2.7^{g}	–	440 ± 1.7^{f}	660 ± 1.0^{g}	550 ± 1.7^{g}	–	660 ± 1.7^{h}	–
2	**MIC**	177 ± 1.8^{f}	252 ± 0.6^{d}	–	101 ± 0.2^{ef}	705 ± 0.3^{k}	252 ± 0.6^{f}	–	378 ± 0.2^{h}	–
	MBC	604 ± 1.3^{i}	504 ± 1.1^{e}	–	214 ± 0.1^{e}	806 ± 0.5^{h}	503 ± 0.2^{f}	–	504 ± 0.1^{f}	–
3	**MIC**	176 ± 0.6^{f}	554 ± 1.2^{h}	–	101 ± 0.3^{f}	504 ± 0.2^{g}	252 ± 0.6^{f}	–	252 ± 0.1^{e}	–
	MBC	504 ± 1.1^{f}	604 ± 1.4^{h}	–	504 ± 0.2^{i}	604 ± 0.1^{f}	504 ± 0.5^{f}	–	504 ± 1.1^{f}	–
4	**MIC**	98 ± 1.7^{c}	25 ± 0.2^{b}	–	98 ± 0.9^{d}	343 ± 0.2^{e}	245 ± 1.0^{e}	–	245 ± 1.7^{d}	–
	MBC	490 ± 1.7^{e}	490 ± 3.3^{d}	–	490 ± 6.6^{g}	490 ± 1.5^{c}	490 ± 1.7^{e}	–	490 ± 1.7^{d}	–
5	**MIC**	345 ± 1.6^{h}	394 ± 0.6^{e}	–	345 ± 0.8^{i}	542 ± 1.5^{h}	394 ± 0.07^{g}	–	492 ± 0.2^{i}	–
	MBC	492 ± 0.9^{e}	492 ± 1.3^{d}	–	492 ± 1.2^{h}	591 ± 2.9^{e}	492 ± 0.1^{e}	492 ± 1.3^{c}	492 ± 0.1^{e}	–
6	**MIC**	101 ± 0.4^{d}	253 ± 0.5^{d}	607 ± 2.4^{c}	253 ± 1.7^{h}	455 ± 0.6^{f}	455 ± 0.3^{i}	–	354 ± 1.4^{g}	–
	MBC	607 ± 0.2^{j}	506 ± 2.0^{e}	–	506 ± 1.0^{i}	607 ± 0.7^{f}	506 ± 1.0^{f}	–	506 ± 2.0^{f}	–
7	**MIC**	356 ± 1.8^{i}	488 ± 2.5^{f}	–	98 ± 0.5^{de}	683 ± 1.6^{j}	244 ± 0.2^{d}	–	244 ± 1.3^{d}	–
	MBC	488 ± 2.5^{d}	537 ± 2.1^{f}	–	488 ± 0.9^{gh}	–	488 ± 1.0^{d}	–	488 ± 0.3^{d}	–
8	**MIC**	94 ± 0.6^{c}	165 ± 0.3^{c}	–	94 ± 0.1^{d}	140 ± 1.7^{c}	24 ± 0.2^{b}	–	112 ± 1.1^{c}	–
	MBC	140 ± 1.7^{c}	140 ± 1.7^{c}	–	165 ± 0.07^{d}	566 ± 0.7^{d}	140 ± 0.7^{c}	–	140 ± 0.7^{c}	–
9	**MIC**	154 ± 0.7^{e}	528 ± 2.7^{g}	–	88 ± 0.7^{c}	616 ± 2.0^{i}	440 ± 0.07^{h}	–	528 ± 2.7^{j}	–
	MBC	528 ± 2.7^{g}	616 ± 2.4^{i}	–	154 ± 0.7^{c}	–	616 ± 2.0^{h}	–	616 ± 2.0^{g}	–
10	**MIC**	24 ± 0.1^{cd}	24 ± 0.1^{c}	49 ± 0.3^{c}	49 ± 0.3^{d}	122 ± 0.3^{e}	122 ± 0.6^{e}	73 ± 0.07^{b}	122 ± 0.7^{e}	–
	MBC	151 ± 0.1^{e}	98 ± 0.2^{d}	195 ± 0.07^{de}	171 ± 0.3^{e}	146 ± 0.1^{d}	146 ± 0.1^{e}	195 ± 0.4^{d}	146 ± 0.4^{d}	–
11	**MIC**	25 ± 0.0^{d}	25 ± 0.2^{c}	49 ± 0.2^{d}	49 ± 0.3^{d}	98 ± 0.7^{d}	98 ± 0.3^{d}	98 ± 0.2^{e}	98 ± 0.7^{d}	–
	MBC	147 ± 2.3^{d}	98 ± 0.3^{d}	147 ± 0.7^{b}	172 ± 0.2^{f}	172 ± 0.2^{e}	172 ± 0.2^{f}	196 ± 0.7^{e}	172 ± 0.6^{e}	–
12	**MIC**	163 ± 0.07^{f}	151 ± 0.2^{f}	163 ± 0.07^{f}	126 ± 0.03^{f}	378 ± 0.2^{f}	378 ± 0.3^{f}	226 ± 0.3^{f}	226 ± 0.6^{f}	–
	MBC	201 ± 0.3^{g}	201 ± 0.3^{e}	201 ± 0.3^{e}	226 ± 0.3^{g}	504 ± 0.2^{f}	504 ± 0.2^{g}	504 ± 0.2^{f}	504 ± 0.1^{f}	–
13	**MIC**	19 ± 0.2^{b}	23 ± 0.07^{b}	93 ± 0.3^{e}	58 ± 0.7^{e}	23 ± 0.07^{b}	46 ± 0.1^{c}	93 ± 0.3^{d}	23 ± 0.6^{bc}	–
	MBC	46 ± 0.1^{c}	46 ± 0.1^{c}	186 ± 0.3^{c}	93 ± 0.3^{d}	46 ± 0.1^{b}	56 ± 0.2^{c}	186 ± 0.2^{c}	46 ± 0.5^{b}	–
14	**MIC**	93 ± 0.3^{e}	46 ± 0.1^{e}	93 ± 0.07^{e}	46 ± 0.1^{c}	23 ± 0.1^{b}	46 ± 0.1^{c}	93 ± 0.07^{d}	23 ± 0.3^{b}	–
	MBC	186 ± 0.2^{f}	58 ± 0.1^{c}	186 ± 1.2^{cd}	70 ± 0.2^{b}	46 ± 0.5^{b}	70 ± 0.2^{d}	186 ± 0.2^{c}	46 ± 0.5^{b}	–
15	**MIC**	13.2 ± 0.07^{ef}	26 ± 0.1^{g}	11 ± 0.2^{c}	5 ± 0.1^{b}	16 ± 0.3^{f}	16 ± 0.07^{e}	11 ± 0.2^{de}	–	13 ± 0.07^{d}
	MBC	42 ± 0.07^{j}	32 ± 0.2^{e}	42 ± 0.07^{h}	11 ± 0.2^{bc}	42 ± 0.1^{h}	42 ± 0.07^{j}	21 ± 0.4^{f}	–	21 ± 0.03^{c}
16	**MIC**	11 ± 0.2^{c}	26 ± 0.5^{g}	11 ± 0.2^{c}	5 ± 0.03^{b}	16 ± 0.3^{f}	16 ± 0.07^{e}	11 ± 0.2^{e}	–	13 ± 0.09^{d}
	MBC	26 ± 0.1^{e}	32 ± 0.2^{e}	21 ± 0.4^{b}	11 ± 0.2^{c}	26 ± 0.1^{b}	26 ± 0.1^{g}	21 ± 0.4^{f}	–	19 ± 0.2^{b}
17	**MIC**	13 ± 0.1^{e}	15 ± 0.07^{d}	5 ± 0.09^{a}	5 ± 0.03^{b}	10 ± 0.07^{b}	10 ± 0.07^{c}	10 ± 0.07^{d}	–	13 ± 0.2^{c}
	MBC	31 ± 0.2^{g}	31 ± 0.2^{e}	41 ± 0.2^{f}	10 ± 0.07^{b}	18 ± 0.3^{a}	13 ± 0.2^{b}	18 ± 0.1^{d}	–	18 ± 0.3^{b}
18	**MIC**	5 ± 0.1^{b}	5 ± 0.03^{a}	5 ± 0.1^{a}	10 ± 0.3^{d}	9 ± 0.1^{a}	10 ± 0.3^{b}	7 ± 0.1^{b}	–	9 ± 0.1^{a}
	MBC	15 ± 0.2^{b}	10 ± 0.1^{a}	15 ± 0.2^{a}	24 ± 0.1^{e}	37 ± 0.2^{f}	24 ± 0.1^{g}	15 ± 0.2^{b}	–	37 ± 0.2^{e}

19	**MIC**	11 ± 0.1^{d}	14 ± 0.2^{c}	9 ± 0.09^{b}	7 ± 0.09^{a}	11 ± 0.1^{c}	16 ± 0.3^{e}	9 ± 0.03^{ab}	–	11 ± 0.1^{b}
	MBC	18 ± 0.07^{c}	16 ± 0.3^{b}	37 ± 0.2^{d}	9 ± 0.2^{c}	37 ± 0.2^{f}	23 ± 0.3^{e}	16 ± 0.3^{c}	–	16 ± 0.05^{a}
20	**MIC**	12 ± 0.2^{d}	14 ± 0.3^{c}	9 ± 0.1^{b}	7 ± 0.1^{a}	23 ± 0.03^{h}	19 ± 0.2^{f}	9 ± 0.1^{c}	–	12 ± 0.2^{b}
	MBC	19 ± 0.2^{c}	28 ± 0.2^{d}	37 ± 0.3^{e}	9 ± 0.2^{e}	28 ± 0.2^{c}	23 ± 0.03^{f}	19 ± 0.2^{e}	–	19 ± 0.2^{b}
21	**MIC**	13 ± 0.03^{ef}	16 ± 0.7^{d}	11 ± 0.2^{c}	11 ± 0.2^{e}	16 ± 0.2^{f}	16 ± 0.2^{e}	11 ± 0.2^{de}	–	13 ± 0.03^{d}
	MBC	31 ± 0.2^{h}	31 ± 0.2^{e}	42 ± 0.03^{gh}	16 ± 0.2^{d}	3 ± 0.1^{d}	19 ± 0.1^{d}	42 ± 0.3^{h}	–	21 ± 0.3^{c}
22	**MIC**	13 ± 0.1^{f}	21 ± 0.2^{e}	16 ± 0.2^{f}	5 ± 0.07^{b}	16 ± 0.1^{f}	16 ± 0.1^{e}	11 ± 0.07^{de}	–	16 ± 0.3^{e}
	MBC	31 ± 0.1^{h}	42 ± 0.1^{g}	42 ± 0.6^{g}	11 ± 0.2^{bc}	31 ± 0.1^{d}	31 ± 0.2^{h}	42 ± 0.03^{h}	–	18 ± 0.1^{b}
23	**MIC**	11 ± 0.1^{d}	14 ± 0.2^{c}	9 ± 0.1^{b}	5 ± 0.2^{a}	14 ± 0.2^{e}	14 ± 0.2^{d}	9 ± 0.2^{c}	–	11 ± 0.1^{b}
	MBC	27 ± 0.3^{f}	27 ± 2.7^{d}	36 ± 0.0^{d}	9 ± 0.2^{a}	27 ± 0.03^{c}	16 ± 0.3^{c}	18 ± 0.1^{de}	–	16 ± 0.3^{a}
24	**MIC**	11 ± 0.1^{d}	23 ± 0.3^{e}	14 ± 0.2^{e}	5 ± 0.3^{a}	14 ± 0.2^{e}	14 ± 0.07^{d}	7 ± 0.1^{a}	–	9 ± 0.1^{a}
	MBC	23 ± 0.2^{d}	27 ± 0.1^{d}	36 ± 0.07^{d}	9 ± 0.1^{a}	27 ± 0.3^{c}	23 ± 0.2^{e}	9 ± 0.07^{a}	–	16 ± 0.3^{a}
25	**MIC**	11 ± 0.1^{d}	16 ± 0.3^{d}	11 ± 0.1^{d}	7 ± 0.3^{c}	14 ± 0.3^{d}	14 ± 0.1^{d}	9 ± 0.7^{c}	–	14 ± 0.2^{d}
	MBC	23 ± 0.2^{d}	23 ± 0.2^{c}	36 ± 0.4^{d}	9 ± 0.2^{a}	36 ± 0.07^{g}	23 ± 0.2^{e}	18 ± 0.03^{de}	–	18 ± 0.03^{b}
Ampi–cilin	**MIC**	25 ± 0.3$^{\mathbf{bc}g}$	25 ± 0.2$^{\mathbf{bd}f}$	25 ± 0.0$^{\mathbf{bb}h}$	37 ± 0.07$^{\mathbf{bb}g}$	37 ± 0.07$^{\mathbf{bc}i}$	25 ± 0.2$^{\mathbf{bb}g}$	74 ± 0.07$^{\mathbf{bc}g}$	25 ± 0.3$^{\mathbf{bc}}$	74 ± 0.3^{g}
	MBC	37 ± 0.07$^{\mathbf{bb}i}$	37 ± 0.07$^{\mathbf{bb}f}$	38 ± 0.02$^{\mathbf{aa}e}$	74 ± 0.1$^{\mathbf{bc}g}$	49 ± 0.07$^{\mathbf{bc}i}$	38 ± 0.07$^{\mathbf{bb}i}$	124 ± 0.6$^{\mathbf{bb}i}$	49 ± 0.07$^{\mathbf{bc}}$	124 ± 0.7^{f}
Strepto–mycin	**MIC**	4 ± 0.1$^{\mathbf{aa}a}$	9 ± 0.2$^{\mathbf{aa}b}$	17 ± 0.02$^{\mathbf{aa}g}$	26 ± 0.3$^{\mathbf{aa}f}$	17 ± 0.07$^{\mathbf{ac}g}$	4 ± 0.0$^{\mathbf{aa}a}$	27 ± 0.1$^{\mathbf{aa}f}$	17 ± 0.07$^{\mathbf{aa}}$	27 ± 0.3^{d}
	MBC	8 ± 0.1$^{\mathbf{aa}a}$	17 ± 0.07$^{\mathbf{aa}b}$	34 ± 0.1$^{\mathbf{aa}c}$	52 ± 0.2$^{\mathbf{aa}f}$	34 ± 0.1$^{\mathbf{ac}e}$	9 ± 0.1$^{\mathbf{aa}a}$	34 ± 0.7$^{\mathbf{aa}g}$	34 ± 0.1$^{\mathbf{aa}}$	34 ± 0.3^{f}

In each line different letters mean significant differences between the compounds ($p < 0.05$).
a–j – letters mean significant differences between the compounds in group 1–9.
a–g – letters mean significant differences between the compounds in group 10–14.
A–j – letters mean significant differences between the compounds in group 15–25.

Table 2 Antifungal activity of XK compounds (MIC and MFC, μmo/ml × 10^{-2}).

Compounds		*A.fum.*	*A.v.*	*A.o.*	*A.n.*	*T.v.*	*P.f.*	*P.o.*	*C.a.*
1	**MIC**	28 ± 0.2^{d}	28 ± 0.2^{c}	55 ± 0.3^{h}	28 ± 0.2^{b}	28 ± 0.2^{d}	28 ± 0.2^{d}	7 ± 0.3^{a}	–
	MFC	165 ± 0.3^{g}	55 ± 0.3^{c}	110 ± 3.3^{f}	165 ± 0.7^{f}	138 ± 0.2^{h}	55 ± 0.3^{b}	28 ± 0.2^{a}	–
2	**MIC**	25 ± 0.03^{c}	6 ± 0.03^{a}	25 ± 0.03^{d}	75 ± 0.1^{h}	25 ± 0.03^{c}	6 ± 0.09^{a}	25 ± 0.03^{b}	200 ± 0.6^{f}
	MFC	100 ± 0.1^{e}	25 ± 0.03^{a}	50 ± 0.07^{b}	100 ± 0.5^{e}	100 ± 0.2^{ef}	25 ± 0.3^{a}	50 ± 0.07^{b}	–
3	**MIC**	25 ± 0.03^{e}	126 ± 0.2^{g}	25 ± 0.03^{d}	75 ± 0.3^{h}	25 ± 0.03^{bc}	6 ± 0.1^{a}	25 ± 0.03^{b}	–
	MFC	75 ± 0.07^{b}	151 ± 0.2^{g}	50 ± 0.3^{b}	100 ± 0.1^{e}	100 ± 0.3^{ef}	25 ± 0.0^{a}	50 ± 0.07^{b}	–
4	**MIC**	25 ± 0.2^{b}	25 ± 0.2^{b}	25 ± 0.2^{c}	74 ± 0.2^{g}	25 ± 0.2^{bc}	6 ± 0.04^{a}	25 ± 0.2^{b}	25 ± 0.03^{b}
	MFC	98 ± 0.3^{d}	49 ± 0.3^{b}	49 ± 0.3^{b}	172 ± 0.2^{g}	98 ± 0.3^{e}	25 ± 0.2^{a}	49 ± 0.3^{b}	123 ± 0.6^{d}
5	**MIC**	25 ± 0.2^{bc}	25 ± 0.2^{b}	6 ± 0.06^{a}	25 ± 0.2^{a}	25 ± 0.1^{bc}	6 ± 0.05^{a}	25 ± 0.2^{b}	–
	MFC	98 ± 0.1^{d}	49 ± 0.07^{b}	25 ± 0.3^{a}	98 ± 0.1^{d}	74 ± 0.6^{b}	25 ± 0.2^{a}	49 ± 0.07^{b}	–
6	**MIC**	76 ± 0.3^{h}	76 ± 0.3^{e}	76 ± 0.3^{j}	76 ± 0.3^{i}	76 ± 0.3^{g}	6 ± 0.1^{a}	6 ± 0.1^{a}	25 ± 0.03^{b}
	MFC	177 ± 0.3^{i}	127 ± 0.2^{f}	177 ± 0.3^{h}	101 ± 0.07^{e}	101 ± 0.3^{f}	76 ± 0.3^{e}	76 ± 0.3^{e}	51 ± 0.06^{b}
7	**MIC**	24 ± 0.1^{b}	6 ± 0.03^{d}	24 ± 0.1^{c}	73 ± 0.07^{g}	24 ± 0.1^{b}	24 ± 0.1^{c}	49 ± 0.3^{c}	24 ± 0.03^{a}
	MFC	171 ± 0.07^{h}	24 ± 0.1^{a}	49 ± 0.3^{b}	171 ± 0.3^{g}	49 ± 0.3^{a}	73 ± 0.07^{d}	98 ± 0.2^{g}	
8	**MIC**	24 ± 0.2^{a}	6 ± 0.03^{a}	24 ± 0.2^{b}	71 ± 0.3^{f}	24 ± 0.2^{a}	24 ± 0.2^{b}	24 ± 0.2^{b}	24 ± 0.1^{a}
	MFC	94 ± 0.1^{c}	24 ± 0.2^{a}	165 ± 0.07^{g}	94 ± 0.1^{c}	118 ± 0.7^{g}	71 ± 0.07^{c}	71 ± 0.3^{d}	–
9	**MIC**	66 ± 0.3^{g}	66 ± 0.2^{e}	66 ± 0.3^{i}	66 ± 0.3^{e}	66 ± 0.0^{f}	66 ± 0.3^{g}	66 ± 0.3^{d}	176 ± 0.6^{e}
	MFC	154 ± 0.7^{f}	88 ± 0.3^{e}	88 ± 0.3^{d}	88 ± 0.3^{b}	88 ± 0.3^{d}	88 ± 0.3^{g}	88 ± 0.0^{f}	–
10	**MIC**	12 ± 0.07^{a}	6 ± 0.03^{a}	24 ± 0.1^{b}	73 ± 0.3^{f}	12 ± 0.07^{a}	6 ± 0.09^{ab}	24 ± 0.1^{b}	171 ± 0.3^{b}
	MFC	98 ± 0.2^{d}	24 ± 0.1^{b}	49 ± 0.4^{b}	98 ± 0.2^{c}	49 ± 0.3^{a}	24 ± 0.1^{b}	98 ± 0.2^{e}	–
11	**MIC**	25 ± 0.2^{b}	25 ± 0.2^{b}	25 ± 0.2^{b}	25 ± 0.2^{a}	25 ± 0.2^{c}	25 ± 0.2^{c}	25 ± 0.2^{b}	–
	MFC	147 ± 0.3^{f}	98 ± 0.3^{d}	147 ± 0.3^{f}	98 ± 0.3^{c}	74 ± 0.2^{b}	49 ± 0.3^{c}	74 ± 0.5^{d}	–
12	**MIC**	51 ± 0.3^{f}	6 ± 0.1^{a}	25 ± 0.03^{c}	75 ± 0.1^{g}	51 ± 0.3^{e}	6 ± 0.1^{b}	25 ± 0.4^{b}	176 ± 0.6^{c}
	MFC	100 ± 0.2^{e}	25 ± 0.4^{b}	51 ± 0.3^{c}	176 ± 0.2^{f}	100. ± 0.3^{e}	51 ± 0.3^{d}	51 ± 0.07^{b}	–
13	MIC	46 ± 0.1^{d}	6 ± 0.3^{a}	6 ± 0.07^{a}	70 ± 0.2^{d}	46 ± 0.1^{d}	6 ± 0.07^{a}	23 ± 0.07^{a}	–
	MFC	93 ± 0.07^{b}	23 ± 0.07^{a}	46 ± 0.1^{a}	162 ± 0.1^{e}	93 ± 0.07^{d}	23 ± 0.4^{a}	46 ± 0.1^{a}	–
14	**MIC**	46 ± 0.5^{a}	6 ± 0.2^{a}	6 ± 0.1^{a}	70 ± 0.03^{e}	23 ± 0.4^{b}	6 ± 0.1^{a}	23 ± 0.4^{a}	–
	MFC	93 ± 0.07^{b}	23 ± 0.3^{a}	46 ± 0.5^{a}	139 ± 0.6^{d}	93 ± 0.3^{d}	23 ± 0.07^{a}	46 ± 0.1^{a}	–
15	**MIC**	13 ± 0.06^{c}	13 ± 0.07^{d}	13 ± 0.06^{d}	13 ± 0.06^{cd}	13 ± 0.06^{e}	13 ± 0.06^{c}	13 ± 0.07^{d}	21 ± 0.4^{d}
	MFC	26 ± 0.1^{g}	26 ± 0.1^{g}	26 ± 0.1^{f}	39 ± 0.2^{i}	21 ± 0.3^{d}	26 ± 0.1^{d}	26 ± 0.1^{e}	40 ± 0.3^{i}
16	**MIC**	13 ± 0.4^{c}	13 ± 0.4^{cd}	13 ± 0.4^{cd}	13 ± 0.1^{cd}	13 ± 0.06^{e}	13 ± 0.4^{c}	13 ± 0.1^{d}	21 ± 0.3^{e}
	MFC	37 ± 0.3^{j}	26 ± 0.5^{g}	26 ± 0.1^{f}	37 ± 0.3^{h}	16 ± 0.3^{c}	26 ± 0.5^{d}	26 ± 0.5^{e}	37 ± 0.3^{h}
17	**MIC**	12 ± 0.2^{c}	13 ± 0.2^{c}	13 ± 0.2^{c}	13 ± 0.1^{c}	13 ± 0.2^{d}	13 ± 0.1^{c}	13 ± 0.2^{c}	20 ± 0.1^{d}
	MFC	36 ± 0.2^{i}	25 ± 0.1^{f}	13 ± 0.2^{c}	25 ± 0.1^{c}	25 ± 0.1^{f}	20 ± 0.1^{b}	25 ± 0.1^{d}	36 ± 0.2^{g}
18	**MIC**	5 ± 0.2^{a}	5 ± 0.2^{a}	5 ± 0.3^{a}	4 ± 0.2^{a}	5 ± 0.03^{a}	5 ± 0.3^{a}	5 ± 0.03^{a}	10 ± 0.3^{a}
	MFC	10 ± 0.3^{a}	10 ± 0.3^{a}	7 ± 0.2^{a}	11 ± 0.07^{a}	7 ± 0.1^{a}	10 ± 0.6^{a}	7 ± 0.1^{a}	20 ± 0.2^{a}
19	**MIC**	11 ± 0.3^{b}	11 ± 0.2^{b}	11 ± 0.1^{b}	11 ± 0.1^{b}	11 ± 0.1^{b}	23 ± 0.3^{d}	11 ± 0.1^{b}	23 ± 0.3^{f}
	MFC	18 ± 0.07^{d}	23 ± 0.3^{de}	18 ± 0.06^{e}	32 ± 0.3^{d}	23 ± 0.3^{e}	32 ± 0.3^{e}	23 ± 0.3^{bc}	32 ± 0.2^{d}
20	**MIC**	12 ± 0.2^{b}	12 ± 0.1^{b}	12 ± 0.2^{b}	23 ± 0.03^{e}	12 ± 0.2^{b}	23 ± 0.03^{d}	12 ± 0.07^{b}	23 ± 0.1^{g}
	MFC	14 ± 0.3^{b}	23 ± 0.03^{e}	14 ± 0.03^{d}	32 ± 0.1^{e}	14 ± 0.3^{b}	32 ± 0.1^{ef}	23 ± 0.03^{c}	32 ± 0.1^{e}
21	**MIC**	13 ± 0.4^{c}	13 ± 0.2^{d}	13 ± 0.4^{cd}	13 ± 0.1^{d}	13 ± 0.4^{de}	13 ± 0.03^{c}	13 ± 0.2^{d}	16 ± 0.2^{c}
	MFC	16 ± 0.2^{c}	37 ± 0.2^{i}	26 ± 0.07^{f}	37 ± 0.2^{h}	37 ± 0.2^{j}	26 ± 0.07^{d}	26 ± 0.07^{e}	26 ± 0.07^{c}
22	**MIC**	13 ± 0.03^{c}	13 ± 0.03^{d}	13 ± 0.03^{d}	13 ± 0.2^{d}	13 ± 0.03^{e}	13 ± 0.4^{c}	13 ± 0.2^{d}	–
	MFC	21 ± 0.3^{e}	16 ± 0.2^{c}	33 ± 0.4^{g}	33 ± 0.03^{f}	33 ± 0.07^{h}	33 ± 0.03^{f}	33 ± 0.2^{f}	–
23	**MIC**	11 ± 0.1^{b}	11 ± 0.1^{b}	11 ± 0.1^{b}	11 ± 0.1^{b}	23 ± 0.2^{f}	11 ± 0.1^{b}	11 ± 0.1^{b}	23 ± 0.2^{f}
	MFC	23 ± 0.2^{f}	23 ± 0.2^{d}	14 ± 0.2^{d}	37 ± 0.2^{h}	32 ± 0.2^{g}	23 ± 0.2^{c}	23 ± 0.2^{b}	32 ± 0.2^{d}
24	**MIC**	11 ± 0.1^{b}	11 ± 0.07^{b}	11 ± 0.1^{b}	11 ± 0.1^{b}	22 ± 0.2^{f}	11 ± 0.2^{b}	11 ± 0.07^{b}	11 ± 0.1^{b}
	MFC	23 ± 0.2^{f}	11 ± 0.1^{b}	11 ± 0.1^{b}	23 ± 0.2^{b}	32 ± 0.2^{g}	23 ± 0.5^{c}	23 ± 0.1^{b}	23 ± 0.2^{b}
25	**MIC**	11 ± 0.2^{b}	11 ± 0.1^{b}	11 ± 0.2^{b}	11 ± 0.1^{b}	11 ± 0.1^{b}	11 ± 0.1^{b}	11 ± 0.1^{b}	32 ± 0.2^{h}
	MFC	32 ± 0.2^{h}	35 ± 0.2^{h}	18 ± 0.4^{e}	35 ± 0.2^{g}	35 ± 0.2^{i}	35 ± 0.2^{g}	35 ± 0.2^{g}	35 ± 0.2^{f}

Table 2 (*continued*)

Compounds		*A.fum.*	*A.v.*	*A.o.*	*A.n.*	*T.v.*	*P.f.*	*P.o.*	*C.a.*
Ketoconazole	**MIC**	$38.0 \pm 0.3^{\mathbf{ec}d}$	$285 \pm 0.3^{\mathbf{hd}f}$	$38 \pm 0.3^{\mathbf{fd}e}$	$38 \pm 0.3^{\mathbf{cb}f}$	$475 \pm 0.7^{\mathbf{hf}h}$	$38 \pm 0.3^{\mathbf{ed}e}$	$380 \pm 3.3^{\mathbf{ec}f}$	$38 \pm 0.03^{\mathbf{da}i}$
	MFC	$95.0 \pm 0.0^{\mathbf{cc}l}$	$380 \pm 2.0^{\mathbf{he}k}$	$95 \pm 0.3^{\mathbf{ee}i}$	$95 \pm 0.3^{\mathbf{cb}k}$	$570 \pm 3.3^{\mathbf{if}l}$	$95 \pm 0.0^{\mathbf{he}i}$	$380 \pm 3.0^{\mathbf{hf}i}$	$94 \pm 0.6^{\mathbf{c}k}$
Bifonazole	**MIC**	$48.0 \pm 0.3^{\mathbf{ee}e}$	$48 \pm 0.0^{\mathbf{dc}e}$	$48 \pm 0.3^{\mathbf{ge}f}$	$48 \pm 0.3^{\mathbf{dc}g}$	$64 \pm 0.3^{\mathbf{eg}g}$	$64 \pm 0.3^{\mathbf{fe}f}$	$48 \pm 0.3^{\mathbf{cd}e}$	$32 \pm 0.0^{\mathbf{ca}h}$
	MFC	$64.0 \pm 0.3^{\mathbf{aa}k}$	$64 \pm 0.3^{\mathbf{dc}j}$	$80 \pm 0.7^{\mathbf{cd}h}$	$64 \pm 0.0^{\mathbf{aa}j}$	$80 \pm 0.3^{\mathbf{cc}k}$	$80 \pm 0.3^{\mathbf{fe}h}$	$64 \pm 0.3^{\mathbf{cc}h}$	$48 \pm 0.6^{\mathbf{a}j}$

In each line different letters mean significant differences between the compounds ($p < 0.05$).
a–j – letters mean significant differences between the compounds in group 1–9.
a–g – letters mean significant differences between the compounds in group 10–14.
a–j – letters mean significant differences between the compounds in group 15–25.

4.3–25.8 · 10^{-2} μmol/mL and MBC of 8.6–51.6 · 10^{-2} μmol/mL. It can be seen that all the compounds tested for antibacterial activity showed better effect than the commercial antibiotic ampicillin with exception of those compounds that did not show any activity in tested concentrations. On the other hand, all the compounds tested exhibited even better antibacterial activity than Streptomycin against *Listeria monocytogenes* and *E. coli* (except for **22** and **21**).

The results of antifungal activity of tested compounds against eight fungi are presented in Table 2. It can be seen that all compounds exhibited antifungal effect. MIC is in range of 3.67–34.6 * 10^{-2} μmol/mL and MFC in 7.35–39.6 * 10^{-2} μmol/mL. The antifungal potential could be presented as: **18 > 24 > 19 > 20 > 23 > 17 > 25 > 16 > 22 > 21 > 15**. Compound **18** again showed the best antifungal activity among other tested compounds with MIC at 3.67–9.80 * 10^{-2} μmol/mL and MFC at 7.35–19.6 * 10^{-2} μmol/mL. Compound **24** is the next one with strong antifungal activity with MIC at 11.3–22.6 * 10^{-2} μmol/mL and MFC 11.3–31.6 * 10^{-2} μmol/mL, while compound **15** possessed the lowest antifungal potential with inhibitory activity at 13.2–21.1 * 10^{-2} μmol/mL and fungicidal activity at 21.1–39.6 * 10^{-2} μmol/mL. The majority of the compounds presented the best activity against *Aspergillus ochraceus, Aspergillus versicolor* and *Aspergillus fumigatus,* while *Candida albicans* was the most resistant species to the compounds. The commercial antifungal agent, bifonazole, showed MIC at 32.0–64.0 * 10^{-2} μmol/mL and MFC at 48.0–80.0 * 10^{-2} μmol/mL. Ketoconazole showed fungistatic activity at 38.0–475.0 * 10^{-2} μmol/mL and fungicidal effect at 95.0–570.0 * 10^{-2} μmol/mL. All the tested compounds showed better fungistatic effect than bifonazole and ketoconazole. Some of the compounds did not exhibit fungicidal activity in tested concentration against *Aspergillus niger, Trichoderma* and *C. albicans*. Compound **18** showed the best effect against bacteria and fungi, while compound **2** exhibited the worst activity against all the tested microorganisms.

It was observed that compounds that exhibited the lowest antibacterial activity are **1–9** (Table 1). The antibacterial potential of this group could be presented as follows: **4 > 8 > 9 > 7 > 5 > 3 > 6 > 1** and **2**. The majority of compounds did not affect some bacteria, such as *M. flavus, E. coli,* and *Enterobacter cloacae*. The compound **4** with highest antibacterial activity showed inhibitory effect at 98.0–490.0 * 10^{-2} μmol/mL bactericidal at 490.0 * 10^{-2} μmol/mL. Compound **2** inhibited bacterial growth with lowest potential at 100.72–377.7 * 10^{-2} μmol/mL, while bactericidal effect was achieved at 214.4–805-76 * 10^{-2} μmol/mL. This compound showed the lowest antibacterial activity among all the others. Streptomycin and Ampicillin showed better antibacterial activity than this group of compounds. Antifungal potential of compounds from this group could be presented as follows: **5 > 4 > 2 > 3 > 8 > 1 > 7 > 9 > 6** (Table 2). Compound **5** showed the best antifungal potential with inhibitory concentration at 6.15–24.6 * 10^{-2} μmol/mL and fungicidal at 24.6–98.4 * 10^{-2} μmol/mL. Compound **4** showed also very good antifungal potential with inhibitory concentration at 6.12–24.5 * 10^{-2} μmol/mL and fungicidal at 49.0–171.5 * 10^{-2} μmol/mL. The lowest antifungal effect was achieved for compound **6** with MIC at 6.3–75.9 * 10^{-2} μmol/mL and MFC at 75.9–177.1 * 10^{-2} μmol/mL. Only compounds **7, 8, 9, 6, 2**, and **4** exhibited antifungal potential against *C. albicans*. The majority of compounds showed higher antifungal potential than commercial fungicides (Table 2).

The next group of compounds are compounds **10–14** (Table 1). They showed higher antimicrobial activity than previous one. The best activity in this group is seen for compounds **13** and **14** with MIC at18.6–92.8 * 10^{-2} μmol/mL × 10^{-2} and 23.2–92.8 * 10^{-2} μmol/mL × 10^{-2} and MBC at 946.4–185.6 * 10^{-2} μmol/mL. Compounds **10** and **11** showed slightly higher antibacterial activity than previous one, while **12** was the less active with MIC and MBC values (125.9–377.7 · 10^{-2} μmol/mL and 200.8–503.6 · 10^{-2} μmol/ml * 10^{-2}). Antifungal activity for these compounds presented in Table 2 could be presented as follows: **10 > 14 > 13 > 11 > 12.** The best antifungal activity is seen for compound **10** which possessed MIC at 6.1–170.8 * 10^{-2} μmol/mL and MFC at 24.4–97.6 * 10^{-2} μmol/mL. Compound **12** as in the case of antibacterial activity showed the worst antifungal potential with MIC at 6.28–175.7 * 10^{-2} μmol/mL and MFC at 25.1–175.7 * 10^{-2} μmol/mL. Almost all compounds in this group showed better antifungal potential than ketoconazole, with exception of compounds **11** and **12** against *A. fumigatus,* compounds **10, 12, 13, 14** against *A. niger* and *C. albicans*. Bifonazole showed lower antifungal effect than compounds from this group toward all fungi, except *A. fumigatus, A. niger* and *C. albicans*.

It can be seen that the third group of compounds **15–25** possessed the highest antibacterial and antifungal potential and did not affect *E. faecalis* at all, and only **22** against *C. albicans*. Second group of compounds (**10–14**) showed lower activity than previous one, and did not exhibit potential on *E. cloacae* and *C. albicans*. The lowest antimicrobial potential could be seen for third group of compounds **1–9** which showed high MIC and MBC/MFC. These compounds did not exhibit activity toward *E. coli* and *E. cloacae,* and *C. albicans* from

fungi. It is obvious that all three groups of compounds showed at first different level of antibacterial and antifungal activity and then they were inactive against different bacterial and fungal species. The reason of such activity could be because of different mechanisms of action of different derivatives influenced by different chemical groups.

2.2.1. Statistical analysis

For each species, three samples were used and all the assays were carried out in triplicate. The results were expressed as mean values and standard errors, and analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This analysis was carried out using the SPSS v. 18.0 software.

2.3. Elucidating the relation of molecular properties to antimicrobial activity

Physically significant descriptors and pharmaceutically relevant properties were predicted using the QikProp software. Then multivariate data analysis was implemented to elucidate the relation between molecular properties to antimicrobial and antifungal activities of the synthesized compounds. Specifically, we based our approach on the "a priori" knowledge, that compound **18** is the optimum against most of the tested strains as it yields the highest antibacterial and antifungal activities. Therefore, the objective was to determine the physically significant descriptors and pharmaceutically relevant properties of any other synthesized compound that resembles its antibacterial and antifungal fingerprints.

For this purpose a PCA model was extracted in order to discern from the scores plot the synthesized compounds that localize near compound **18** and identify from the loading plot the molecular properties that mainly characterize them. The PCA model ($A = 2$, $N = 25$, $R^2X(cum) = 0.92$, $Q^2(cum) = 0.83$) was extracted based only on the physicochemical properties of the synthesized compounds (Fig. 1). The formation of two groups is evident along the PC1 principal component. Compound **18** belongs to Group 1 together with **17, 5, 10, 11, 4, 7, 9,19, 8, 6, 16** and **17**. This suggests that these molecules probably exhibit similarly potent biological activities explained by some common descriptors. The loading plot highlights the descriptors which contribute to the clustering between the two groups. From these, the most significant are the (a) number of non-trivial (not CX3), non-hindered (not alkene, amide, small ring) rotatable bonds (#rotor), (b) total

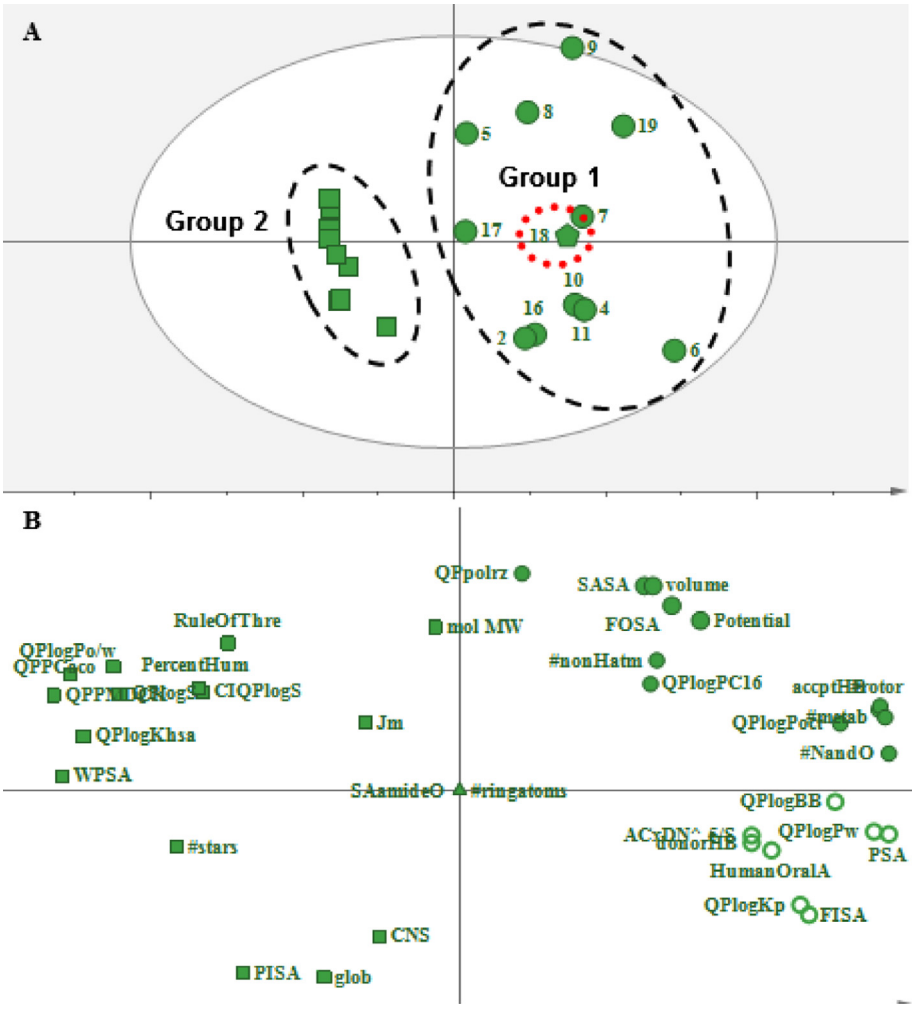


Figure 1 PCA model $A = 2$, $N = 25$, $R^2X(cum) = 0.92$, $Q^2(cum) = 0.83$. A. Scores scatter plot depicting a clear separation into two groups (Group1: circles; Group 2: squares) B. Loading scatter plot displaying the variables that characterize each group.

solvent accessible surface area (SASA) in square angstroms using a probe with a 1.4 A radius, (c) hydrophobic component of the SASA(FOSA), (d) hydrophilic component of the SASA (FISA), (e) total solvent-accessible volume in cubic angstroms using a probe with a 1.4 Å radius (volume), (f) estimated average number of hydrogen bonds (taken over a number of configurations) that would be donated by the solute to water molecules in an aqueous solution (HBd), (g) estimated average number of hydrogen bonds (taken over a number of configurations)that would be accepted by the solute from water molecules in an aqueous solution (HBa), (h) hexadecane/gas partition coefficient (QPlogPC16), (i) octanol/gas partition coefficient (QPlogPoct), (j) water/gas partition coefficient (QPlogPw), (k) predicted brain/blood partition coefficient (QPlogBB), (l) predicted skin permeability (QPlogKp), (m) number of likely metabolic reactions (#metab), (n) Van der Waals surface area of polar nitrogen and oxygen atoms and carbonyl carbon atoms (PSA), (o) number of nitrogen and oxygen atoms (#NandO), (p) number of heavy atoms (#nonHatm). These produced values can be further used as descriptors for QSAR and *in silico* screening techniques.

2.4. Generation of pharmacophore model for antifungal activity

The generation of the pharmacophore model using a training set of six compounds (**8, 16, 18, 19, 23** and **25**) resulted in top ten hypotheses with individual ranking scores and pharmacophore features as depicted in Table 3 for *A. fumigatus* and *C. albicans*.

Table 3 The pharmocophoric features generated.

Hypo	Feature[a]	Rank[b]	Direct hit[c]	Partial hit[d]
A. fumigatus				
1	RHHAAA	94.84	101111	010000
2	RHHAAA	94.84	101111	010000
3	RHHAAA	94.28	101111	010000
4	RHHAAA	93.99	101111	010000
5	RHHAAA	91.68	101111	010000
6	RHHAAA	90.94	101111	010000
7	RHHAAA	90.15	101111	010000
8	RHHAAA	88.52	101111	010000
9	RHHAAA	88.52	101111	010000
10	RHHAAA	87.3	101111	010000
C. albicans				
1	RHHAAA	94.84	101111	010000
2	RHHAAA	94.84	101111	010000
3	RHHAAA	92.10	101111	010000
4	RHHAAA	90.94	101111	010000
5	RHHAAA	88.83	101111	010000
6	RHHAAA	87.42	101111	010000
7	RRHHA	81.14	101111	010000
8	RRHHA	81.14	101111	010000
9	RRHHA	81.14	101111	010000
10	RHHAA	80.51	101111	010000

[a] R = Ring Aromatic group; H = Hydrophobic aliphatic group; A = Hydrogen bond acceptor group.
[b] Higher ranking score indicate less probability that the molecules in the training set fit the hypothesis by a chance correlation. The best hypotheses have the highest ranking score.
[c] Direct Hit = all the features are mapped. Direct Hit = 1 means yes.
[d] Partial Hit = partial mapping of the hypothesis. Partial Hit = 0 means No.

It was interesting to note that the top two hypothesis (Hypo1 and Hypo2) in both species (*A. fumigatus* & *C. albicans*) had exactly the same pharmacophoric features (RHHAAA) suggesting possibly the same target in both these species. Both these hypothesis (hypo1 and Hypo2) in both species *(A. fumigatus* & *C. albicans)* also showed same ranking score of 94.84 and were constituted by two HAl(HAl1 and HAl2), three HBA(HBA1, HBA2 and HBA3) and one (RA) features. All the ten generated hypotheses in case of *A. fumigatus* were found to be uniform regarding the composition of chemical features. However in case of *C. albicans* the first five hypotheses were found to be uniform in terms of pharmacophore feature composition. The Hypo1 for both *A. fumigatus* and *C. albicans* having same chemical feature composition (RHHAAA) showed similar chemical feature mapping for the most active compound **18**. The preliminary pharmacophore models in both the species (*A. fumigatus* and *C. albicans*) were further refined by addition of excluded volumes for identification of the least active compounds in the series properly. As an optimization strategy the HAl1 feature was also modified to have a location sphere radius of 2.5 Å in both cases (*A. fumigatus* and *C. albicans*) to make improvements in prediction. The location sphere radius for the excluded volumes were optimized and reduced to lower values so as to assign better fit values for the active compounds and lower fit values for the least active compounds in the series. In both CFPM compound **18** (Fig. 2a & c) was observed to map the HAl1 with the 3-methoxy group attached to the phenyl ring while the adamantyl group present at the other terminal mapped the HAl2 feature. The threes HBA features generated mapped the thiazole sulfur atom (HBA1), the ketooxygen atom (HBA2) and the triazole nitrogen atom (HBA3) while the RA feature generated mapped the phenyl ring of compound **18** (Fig. 2a & c). The distance matrix of pharmacophore features for Hypo1 for both *A. fumigatus* and *C. albicans* were found to be the same and has been presented in supporting information (Table 3). The least active compound **6** in the series of *A. fumigatus* mapped all the features of the pharmacophore (CFPM of *A. fumigatus*) with the same functional groups as observed for compound **18** except HAl1 as the compound lacks a hydrophobic feature at this position (Fig. 2b). A similar mismatch with the HAl1 was also observed for the least active compound **2** in the series for *C. albicans* (CFPM of *C. albicans*) (Fig. 2d). The pharmacophore fit values (Supplementary information: Table 2) obtained from the CFPM were further correlated with biological activity (pMIC) of the compounds for each species. The generated CFPM for *A. fumigatus* was found to have a correlation value (R) of 0.51(n = 25) between observed activity (pMIC) and pharmacophore fit values that improved to 0.74 (n = 23) when two outliers (**5, 23**) were removed from the series (Fig. 2e). In case of *C. albicans* similar improvement (n = 18, R = 0.48; n = 16, R = 0.74) (Fig. 2f) was observed when two outliers (**20, 23**) were removed from the series that indicate the generated pharmacophore models have good ability to predict the individual antifungal activity in these series of compounds.

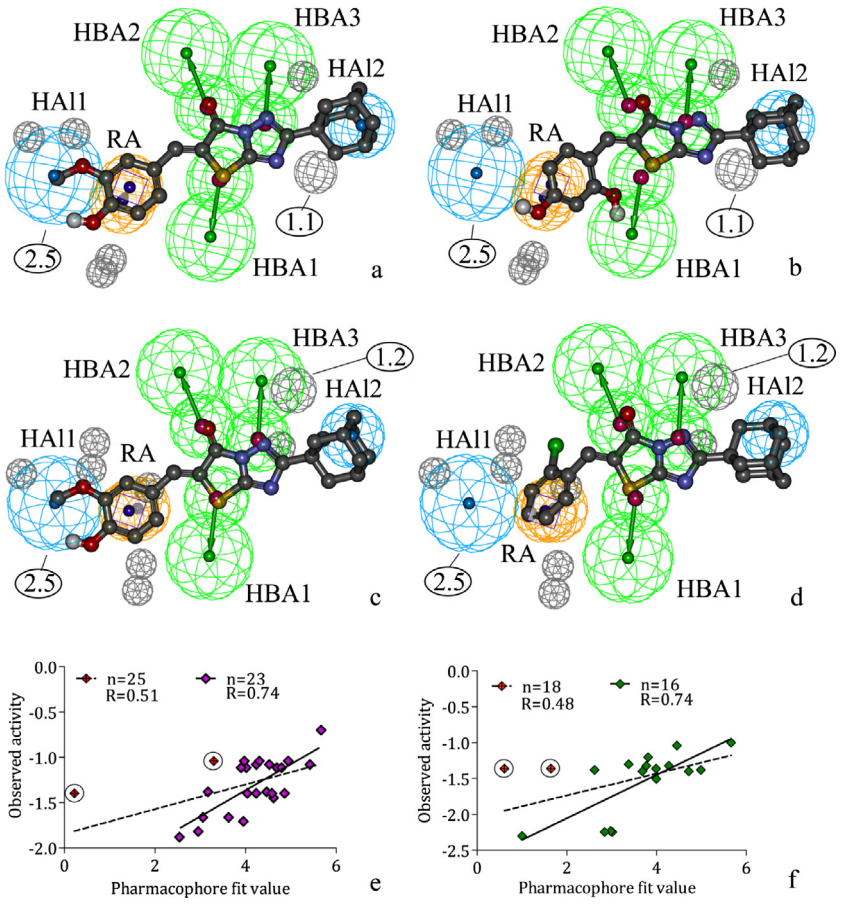


Figure 2 (a). Mapping of the most active compound **18** with the CFPM of *A. fumigatus*. (b) Mapping of the least active compound **6** with the CFPM of *A. fumigatus*. (c) Mapping of the most active compound **18** with the CFPM of *C. albicans*. (d) Mapping of the least active compound **18** with the CFPM of *C. albicans*. (e) Graph displaying correlation between observed activity (pMIC) and pharmacophore fit value for (e) *A. fumigatus* (f) *C. albicans*. All the excluded volumes have location sphere of radius of 0.8 Å except those mentioned in the figure. The HAl1 has a location sphere of radius 2.5 Å.

3. Conclusions

In summary this study proposes twenty-five novel compounds as putative novel antimicrobials. Particularly, a series of novel 5-benzylideno-2-adamantylthiazol [3,2-*b*][1,2,4]triazol-6(5*H*)ones were synthesized and evaluated *in vitro* for their antimicrobial properties against Gram positive, Gram negative bacteria and fungi strains.

Almost all the tested compounds showed antibacterial activity but on different level and this activity was even better than that of Streptomycin against *L. monocytogenes,* and *E. coli*. All compounds showed antifungal effect with MIC in range of 3.67–34.6 $*$ 10^{-2} μmol/mL and MFC in 7.35–39.6 $*$ 10^{-2} μmol/mL. The majority of the compounds showed the best activity against *A. ochraceus, A. versicolor* and *A. fumigatus,* while *C. albicans* was the most resistant species to the compounds. All the compounds tested showed better fungistatic effect than bifonazole and ketoconazole. It was observed that the best antifungal activity was observed for compounds **15–25**. Compounds **10–13** and **14** were less active than the previous ones, while the lowest activity was expressed by the rest of compounds. The application of multivariate data analysis suggested the physically significant descriptors and properties that result in the optimum antibacterial and antifungal fingerprints for the examined compounds. In light of this, the highlighted parameters can be further used as descriptors for QSAR and *in silico* screening techniques.

Moreover, for *A. fumigatus* and *C. albicans* the generation of pharmacophore was performed. It was interesting to note that both species *(A. fumigatus* & *C. albicans)* had exactly the same pharmacophoric features (RHHAAA) suggesting possibly the same target in both these species. The results indicated that generated pharmacophore models have good ability to predict the individual antifungal activity in these series of compounds in case of *C. albicans*.

4. Experimental section

4.1. Chemistry-general aspects

Melting points (°C) were determined with a MELTEMP II capillary apparatus (LAB Devices, Holliston, MA, USA) without correction. Elemental analyses were performed on a Perkin–Elmer 2400 CHN elemental analyzer and for all compounds synthesized were within a 0.4% of theoretical values. IR spectra were recorded, as Nujol mulls, on a Perkin Elmer Spectrum BX. Wave numbers in the IR spectra are given in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra of the newly synthesized compounds, in DMSO-d_6 or $CDCl_3$ solutions, were recorded on a Bruker AC 300 instrument (Bruker, Karlsruhe, Germany) at 298 K. Chemical shifts are reported as δ (ppm) relative to TMS as internal standard. Coupling constants J are expressed in Hertz (Center of Instrumental Analysis of the University of Thessaloniki). The reactions were monitored by TLC on F_{254} silica-gel precoated sheets (Merck,

Darmstadt, Germany) and each of the purified compounds showed a single spot. Solvents, unless otherwise specified were of analytical reagent grade or of the highest quality commercially available. Synthetic starting materials, reagents and solvents were purchased from Aldrich Chemie (Steinheimm, Germany).

4.2. Synthesis of 1-(1-adamantanocarbonylo)thiosemicarbazide (Tozkoparan et al., 2000)

To a solution of thiosemicarbazide (0.1 mol, 9.1 g) in dry pyridine (150 ml), a solution of 1-adamantylcrabonyl chloride (0.1 mol, 19.869 g) in dry benzene (150 ml) under stirring and temperature at −5 °C was added. The reaction mixture was stirred for another 0.5 h at −5 °C and after left at room temperature for 12 h. After removal of solvents, to the remain solid water was added and precipitate was filtered and recrystallized from methanol: dichloromethane. Yield: 70%.

4.3. Synthesis of 5-adamantyl-4H-1,2,4-triazol-3-thiol ((Tozkoparan et al., 2000)

To a solution of NaOH 5% (5 ml) 1-(1-adamantanocarbonylo) thiosemicarbazide (0.01 mol) was added and reaction mixture was refluxed for 2 h. After cooling to reaction mixture HCl 10% was added till pH = 6. The precipitate washed with water and filtered. Dry product recrystallized from methanol. Yield, 93%.**IR**: (cm^{-1}, Nujol): 3554 (NH), 2670 (SH), 1579 (C═N). **^{1}H NMR:** (δ ppm, DMSO-d_6, 300 MHz): 1.70–2.51 (m, 15H, adamantane).

4.4. General method for preparation of 5-aryliden -2-adamatylthiazol[3,3-b]triazol-6(5H)-ones (Vicini et al., 2008)

To a solution of 5-adamantyl-4*H*-1,2,4-triazol-3-thiol (4 mol) in acetic acid (10 ml), bromoacetic acid (6 mol), appropriate aromatic aldehyde (4 mol), sodium acetate (0.54 gr, 6.58 mmol) and acetic anhydride (8 ml) were added. The reaction mixture was refluxed under stirring for 6 h. The obtained product left for some hours at room temperature and after pawed to the ice. The obtained precipitate filtered and recrystallized from dioxane.

*4.4.1. 2-Adamantyl-5-(benzyliden)thiazol[3,2-b][1,2,4]triazol-6(5H)-one (**1**)*

Yield: 42%, m.p. 203–205 °C, R_f = 0.63 (toluene-ethanol 8:2), ^{1}H NMR:(DMSO-d_6) δ (ppm): 1.75–2.05 (m, 15H, adamantan),7.59–7.61 (m, 2H, Ar—H), 7.74–7.77 (m, 3H, Ar—H), 8.22 (s, 1H, CH═). MS (*m*/*z*, I%): (M^+ + 1) 364 (12%), 356 (76%), 330 (100%), 314 (68%), 284 (20%), 268 (36%), 184 (16%), 165 (24%), 125 (16%), 107 (24%). Anal. Calc. for $C_{21}H_{21}N_3OS$ (MW 363): C: 69.39; H: 5.82; N: 11.56%. Found: C: 69.41; H: 5.80; N: 11.53%.

*4.4.2. 2-Adamantyl- 5-(3-hydroxybenzyliden)thiazol[3,2-b][1,2,4]triazol-6(5H)-one (**2**)*

Yield: 63%, m.p. 239–241 °C (dioxan), R_f = 0.75 (toluene-ethanol 8:2), IR:(cm^{-1}, Nujol): 1734 (C═O), 1608 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.82–2.36 (m, 15H, adamantan), 7.28 (s, 1H, Ar—H), 7.35–7,38 (m, 1H, Ar—H), 7.46–7,49 (m, 1H, Ar—H), 7.53–7.58 (m, 1H, Ar—H), 8.16 (s, 1H, CH═). Anal. Calcd. for $C_{21}H_{21}N_3O_2S$(MW 379): C: 66.47; H: 5.58; N: 11.07%. Found: C 66.61; H 6.00; N: 10.98%.

*4.4.3. 2-Adamantyl -5-(4-hydroxybenzyliden)thiazol[3,2-b][1,2,4]triazol-6(5H)-one (**3**)*

Yield: 80%, m.p. 238–240 °C (ethanol), R_f = 0.7 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol): 1736 (C═O), 1595 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.76–2.31 (m, 15H, adamantan), 7.38 (d, J = 6 Hz, 2H, Ar—H), 7.80 (d, J = 9 Hz, 2H, Ar—H), 8.22 (s, 1H, CH═). **MS:** (*m*/*z*): 379 (M^+, 18%), 378 (100%), 285 (4%), 283 (53%), 255 (60%), 241 (6%), 235 (19%), 234 (86%), 233 (31%), 218 (3%), 191 (3%). Anal. Calcd for $C_{21}H_{21}N_3O_2S$ (MW 379):C: 66.47, H: 5.58, N: 11.07%. Found: C:66.55; H:5.56; N: 11.02%.

*4.4.4. 2-Adamantyl-5-(4-methoxybenzyliden)thiazol[3,2-b][1,2,4]triazol-6(5H)-one (**4**)*

Yield: 71%, m.p. 250–252 °C (ethanol), R_f = 0.31 (toluene-ethanol 9.5:0.5), IR: (cm^{-1}, Nujol): 1734 (C═O), 1608 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.76–2.07 (m, 15H, adamantan), 3.88 (s, 3H, OCH_3), 7.18 (d, J = 9 Hz, 2H, Ar—H), 7.74 (d, J = 9 Hz, 2H, Ar—H), 8.19 (s,1H, CH═). Anal. Calcd. for$C_{22}H_{23}N_3O_2S$: C: 67.15; H: 5.89; N: 10.68%. Found: C: 67.26; H: 5.92; N: 10.65%.

*4.4.5. 2-Adamantyl-5-(2,4-dihydroxybenzyliden)thiazol[3,2-b][1,2,4]triazol-6(5H)-one (**5**)*

Yield: 38%, m.p. 230–232 °C (ethanol), R_f = 0.66 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol): 1739 (C═O), 1608 (C═N).^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.72–2.04 (m, 15H, adamantan), 8.34 (s,1H, CH═). MS (*m*/*z*, I%): (M^+ + 1) 396 (11%), 387 (18%), 386 (100%), 229 (17%), 203 (22%),165 (67%), 157 (17%), 135 (39%). Anal. Calcd. for $C_{21}H_{21}N_3O_3S$ (MW 395):C: 63.78; H: 5.35; N: 10.62%. Found: C: 63.82; H: 5.40; N: 10. 60%.

*4.4.6. 2-Adamantyl-5-(2-hydroxy-3-methoxybenzyliden)thiazol[3,2-b][1,2,4]triazol-6(5H)-one (**6**)*

Yield: 55%, m.p. 234–236 °C (ethanol), R_f = 0.63 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol): 1733 (C═O), 1607 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1,75–2.03 (m, 15H adamantan), 4.01 (s, 3H, OCH_3),7.10–7.17 (m, 2H, Ar—H), 7.25–7.30 (m, 1H, Ar—H). Anal. Calcd. for $C_{22}H_{23}N_3O_3S$ (MW 409): C: 64.53; H: 5.66; N:10.26%. Found:C: 64.60; H: 5.51; N: 10.31%.

*4.4.7. 2-Adamantyl-5-(4-hydroxy-3-methoxybenzyliden)thiazol[3,2-b][1,2,4]triazol-6(5H)-one (**7**)*

Yield: 58%, m.p. 263–265 °C (ethanol), R_f = 0.67 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol):) 1724 (C═O), 1578 (C═N. ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.77–2,30 (m, 15H, adamantan), 3.87 (s, 3H, OCH_3), 7.35 (s, 2H, Ar—H), 7.54 (s, 1H, Ar—H), 8.23 (s, 1H, CH═).

MS: (*m*/*z*): 409 (M^+, 1,3%), 408 (8%), 393 (~2%), 255 (7%), 234 (100%), 235 (15%), 233 (31%), 207 (4%), 2020 (2.5%). Anal. Calcd. for $C_{22}H_{23}N_3O_3S$ (MW): C: 64.53; H: 5.66; N: 10.26%. Found: C: 64.50; H: 5.7; N: 9.98%.

*4.4.8. 2-Adamantyl-5-(3,4-dimethoxybenzyliden)thiazol[3,2-b][1,2,4]triazol-6(5H)-one (**8**)*

Yield: 62%, m.p. 249–251 °C (ethanol), R_f = 0.56 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol): 1723 (C═O), 1590 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1,75–2,05 (m, 15H, adamantan), 3.85 (s, 6H, OCH_3), 7,20 (s, 1H, Ar—H), 7.35–7.38 (m, 2H, Ar—H), 8.18 (s, 1H, CH═).MS (*m*/*z*, I%): (M^+ + 1) 424 (40%), 165 (100%), 135 (30%). Anal. Calcd.for $C_{23}H_{25}N_3O_3S$ (MW 423):C: 62.23; H: 5.95; N: 9.92%. Found: 62.35, H: 5.55, N: 9.82%.

*4.4.9. 2-Adamantyl-5-(4-hydroxy-3,5-dimethoxybenzyliden) thiazol[3,2-b][1,2,4]triazol-6(5H)-one (**9**)*

Yield: 88%, m.p. 263–265 °C (ethanol), R_f = 0.67 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol):) 1724 (C═O), 1578 (C═N. ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.77–2.30 (m, 15H, adamantan), 3.87 (s, 3H, OCH_3), 7.35 (s, 2H, Ar—H), 7.54 (s, 1H, Ar—H), 8.23 (s, 1H, CH═). Anal. Calcd. for $C_{23}H_{25}N_3O_4S$ (MW 439): C: 62.85; H:5.73; N: 9.56%. Found: C: 62.92; H: 5.80; N: 9.61%.

*4.4.10. 2-Adamantyl-5-(3,4,5-trimethoxybenzyliden)thiazol [3,2-b][1,2,4]triazol-6(5H)-one (**10**)*

Yield: 67%, m.p. 305–307 °C (ethanol), R_f = 0.57 (toluene-ethanol 8:2), IR:(cm^{-1}, Nujol):) 1729 (C═O), 1603 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.59–2.11 (m, 15H, adamantan), 3.94 (s, 9H, OCH_3), 6.85 (s, 2H, Ar—H), 8.12 (s, 1H, CH═). MS (*m*/*z*, I%): (M^+ + 1) 409 (27%), 357 (8%), 356 (26%), 338 (26%), 317(24%), 316 (100%), 314 (43%), 298 (18%), 294 (39%), 293 (37%), 276 (74%), 248 (43%), 204 (13%), 174 (44%), 165 (38%), 135 (13%), 125 (39%). Anal. Calcd. for $C_{24}H_{27}N_3O_4S$ (MW 453):C: 63.56; H: 6.0; N: 9.26%. Found: C: 63.49; H: 6.21; N: 9.32%.

*4.4.11. 2-Adamantyl-5-(4-dimethylaminobenzyliden)thiazol [3,2-b][1,2,4]triazol-6(5H)-one (**11**)*

Yield: 69%, m.p. 267–268 °C (ethanol), R_f = 0.63 (toluene-ethanol 8:2), IR:(cm^{-1}, Nujol):) 1728 (C═O), 1586 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.75–2.05 (m, 15H, adamantan), 3.07 (s, 6H, CH_3), 6.87 (d, J = 9 Hz, 2H, Ar—H), 7.60 (d, 2H, J = 8.7 Hz, Ar—H), 8.08 (s, 1H, CH═). MS (*m*/*z*, I%): (M^+ + 1) 409 (41%), 165 (100%), 135 (59%). Anal. Calcd. for $C_{23}H_{26}N_4OS$ (MW 406): C: 67.95; H: 6.45; N: 13.78%. Found: C: 68.02; H: 6.28; N: 13.55%.

*4.4.12. 5-(2-nitrobenzyliden)thiazol[3,2-b][1,2,4]triazol-6 (5H)-one (**12**)*

Yield: 47%, m.p. 159–161 °C (ethanol), R_f = 0.68 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol):) 1749 (C═O), 1625 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$300 MHz): 1.75–2.06 (m, 15H, adamantan), 7.79–7.93 (m, 2H, Ar—H), 7.95–7.98 (m, 3H, Ar—H), 8.29 (d, 1H, J = 8.1 Hz, Ar—H), 8.49 (s, 1H, CH═). MS (*m*/*z*, I%): (M^+ + 1) 398 (5%), 338 (56%), 304 (32%), 248 (40%), 165 (100%), 135 (60%). Anal. Calcd. for $C_{21}H_{20}N_4O_8S$ (MW 406): C: 61.75; H: 4.94; N: 13.72%. Found: C: 61.91; H: 5.02; N: 13. 57%.

*4.4.13. 5-(3-nitrobenzyliden)thiazol[3,2-b][1,2,4]triazol-6 (5H)-one (**13**)*

Yield: 49%, m.p. 205–207 °C (ethanol), R_f = 0.78 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol):) 1739 (C═O), 1608 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.76–2.07 (m, 15H, adamantan), 7.87–7.92 (m, 1H, Ar—H),8.14 (d, 1H, J = 7.8 Hz, Ar—H), 8.355 (s, 1H, Ar—H), 8.36 (s, 1H, CH═), 8.59 (s, 1H, Ar—H). Anal. Calcd. for $C_{21}H_{20}N_4O_8S$ (MW 406): C: 61.75; H: 4.94; N: 13.72%. Found: C: 61.86; H:4.87; N: 13. 68%.

*4.4.14. 5-(4-nitrobenzyliden)thiazol[3,2-b][1,2,4]triazol-6 (5H)-one (**14**)*

Yield: 59%, m.p. 176–178 °C (ethanol), R_f = 0.36 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol):) 1718 (C═O), 1608 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.7–2.0 (m, 15H, adamantan), 7.94 (d, 2H, J = 8.7 Hz, Ar—H), 8.01 (s, 1H, CH═), 8.28 (d, J = 8.7 Hz, 2H, Ar—H). Anal. Calcd. for $C_{21}H_{20}N_4O_8S$ (MW 406): C: 61.75; H: 4.94; N: 13.72%. Found: C: 61.78; H:4.90; N: 13. 70%.

*4.4.15. 5-(3-fluorobenzyliden)thiazol[3,2-b][1,2,4]triazol-6 (5H)-one (**15**)*

Yield: 71%, m.p. 193–195 °C (ethanol), R_f = 0.46 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol):) 1740 (C═O), 1612 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.75–2.06 (m, 15H, adamantan), 7.40–7.46 (m, 1H, Ar—H), 7.59–7.70 (m, 3H, Ar—H), 8.22 (s, 1H, CH═). Anal. Calcd. for $C_{21}H_{20}FN_3OS$: C: 66.12; H: 5.28; N: 11.02%. Found: 66.42; H: 5.7; N: 10.93%.

*4.4.16. 5-(4-fluorobenzyliden)thiazol[3,2-b][1,2,4]triazol-6 (5H)-one (**16**)*

Yield: 66%, m.p. 238–240 °C (ethanol), R_f = 0.73 (toluene-ethanol 9:1), IR:(cm^{-1}, Nujol): 1737 (C═O), 1595 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.77–2.12 (m, 15H, adamantan), 7.22–7.25 (m, 2H, Ar—H), 7.60–7.65 (m, 2H, Ar—H), 8.17 (s, 1H, CH═). Anal. Calcd. for $C_{21}H_{20}FN_3OS$: C: 66.12; H: 5.28; N: 11.02%. Found: 65.99; H: 5.23; N: 10.82%.

*4.4.17. 5-(2-chlorobenzyliden)thiazol[3,2-b][1,2,4]triazol-6 (5H)-one (**17**)*

Yield: 48%, m.p. 246–248 °C (ethanol), R_f = 0.64 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol): 1743 (C═O), 1607 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.82–2.11 (m, 15H, adamantan), 7.43–7.48 (m, 1H, Ar—H), 7.54–7.57 (m, 1H, Ar—H), 7.64–7.67 (m, 1H, Ar—H), 8.54 (s, 1H, CH═). Anal. Calcd. for $C_{21}H_{20}ClN_3OS$ (MW 3.97): C:63.39; H: 5.07; N: 10.56%. Found: C: 63.55; H: 5.13; N: 10.49%.

*4.4.18. 5-(3-chlorobenzyliden)thiazol[3,2-b][1,2,4]triazol-6 (5H)-one (**18**)*

Yield: 47%, m.p. 193–195 °C (ethanol), R_f = 0.57 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol): 1740 (C═O), 1607 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.76–2.06 (m, 15H, adamantan), 7.65–7.70 (m, 3H, Ar—H), 7.83 (s, 1H, Ar—H), 8.22 (s, 1H, CH═). Anal. Calcd. for $C_{21}H_{20}ClN_3OS$ (MW 397): C:63.39; H: 5.07; N: 10.56%. Found: C: 63.45; H: 5.12; N: 10.60%.

*4.4.19. 5-(4-chlorobenzyliden)thiazol[3,2-b][1,2,4]triazol-6 (5H)-one (**19**)*

Yield: 51%, m.p. 242–244 °C (ethanol), R_f = 0.69 (toluene-ethanol 9:1), IR: (cm^{-1}, Nujol): 1731 (C═O), 1604 (C═N).

^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz):1.75–2.06 (m, 15H, adamantan), 7.68 (d, J = 8.7 Hz, 2H, Ar—H), 7.77 (d, J = 8.4 Hz, 2H, Ar—H), 8.23 (s, 1H, CH═). Anal. Calcd. for $C_{21}H_{20}ClN_3OS$ (MW 397): C:63.39; H: 5.07; N: 10.56%. Found: C: 63.41; H: 5.04; N: 10.50%.

4.4.20. 5-(2,3-dichlorobenzyliden)thiazol[3,2-b][1,2,4]triazol-6(5H)-one (**20**)

Yield: 87%, m.p. 201–203 °C (ethanol), R_f = 0.53 ($CHCl_3$), IR: (cm^{-1}, Nujol): 1739 (C═O), 1654 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.82–2.11 (m, 15H, adamantan), 7.39–7.45 (m, 1H, Ar—H), 7.54 (d, J = 7,8 Hz, 1H, Ar—H), 7.64 (d, J = 8,1 Hz, 1H, Ar—H), 7.75 (s,1H, Ar—H), 8.11 (s, 1H, CH═). Anal. Calcd. for $C_{21}H_{19}Cl_2N_3OS$ (MW432):C: 58.34, H: 4.43, N: 9.72%. Found:C: 58.42, H: 4.41, N: 9.65%.

4.4.21. 5-(2,4-dichlorobenzyliden)thiazol[3,2-b][1,2,4]triazol-6(5H)-one (**21**)

Yield: 51%, m.p. 203–205 °C (ethanol), R_f = 0.53 (toluene-ethanol 8:2), IR: (cm^{-1}, Nujol): 1740 (C═O), 1578 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.82–2.10 (m, 15H, adamantan), 7.41–7.48 (m, 1H, Ar—H), 7.54–7.62 (m, 2H, Ar—H), 8.45 (s, 1H, CH═). Anal. Calcd. for $C_{21}H_{19}Cl_2N_3OS$ (MW432): C: 58.34, H: 4.43, N: 9.72%. Found:C: 58.30, H: 4.46, N: 9.68%.

4.4.22. 5-(2,6-dichlorobenzyliden)thiazol[3,2-b][1,2,4]triazol-6(5H)-one (**22**)

Yield: 53%, m.p. 203–205 °C (ethanol), R_f = 0.74 (toluene-ethanol 8:2), IR:(cm^{-1}, Nujol): 1751 (C═O), 1628 (C═N). Anal. Calcd. for $C_{21}H_{19}Cl_2N_3OS$ (MW432):C: 58.34, H: 4.43, N: 9.72%. Found: C: 58.29, H: 4.38, N: 9.73%.

4.4.23. 5-(2-bromobenzyliden)thiazol[3,2-b][1,2,4]triazol-6 (5H)-one (**23**)

Yield: 55%, m.p. 249–251 °C (dioxan), R_f = 0.43 ($CHCl_3$), IR: (cm^{-1}, Nujol): 1742 (C═O), 1607 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz):1.82–2.11 (m, 15H, adamantan), 7.32–7.38 (m, 1H, Ar—H), 7.46–7.51 (m, 1H, Ar—H), 7.62 (d, J = 7,8 Hz, 1H, Ar—H), 7.74 (d, J = 7,95 Hz, 1H, Ar—H), 8.48 (s, 1H, CH═). Anal. Calcd. for $C_{21}H_{20}BrN_3OS$: C: 57.02, H: 4.56, N: 9.50%. Found: C: 56.75, H: 4.98, N: 9.07%.

4.4.24. 5-(3-bromobenzyliden)thiazol[3,2-b][1,2,4]triazol-6 (5H)-one (**24**)

Yield: 79%, m.p. 191–193 °C (dioxan), R_f = 0.53 ($CHCl_3$), IR: (cm^{-1}, Nujol): 1739 (C═O), 1654 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.82–2.11 (m, 15H, adamantan), 7.39–7.45 (m, 1H, Ar—H), 7.54 (d, J = 7,8 Hz, 1H, Ar—H), 7.64 (d, J = 8,1 Hz, 1H, Ar—H), 7.75 (s, 1H, Ar—H), 8.11 (s, 1H, CH═). Anal. Calcd.for $C_{21}H_{20}BrN_3OS$: C: 57.02, H: 4.56, N: 9.50%. Found: C: 57.35, H: 4.81, N: 9.13%.

4.4.25. 5-(4-bromobenzyliden)thiazol[3,2-b][1,2,4]triazol-6 (5H)-one (**25**)

Yield: 75%, m.p. 201–2033 °C (ethanol), R_f = 0.41 ($CHCl_3$), IR:(cm^{-1}, Nujol): 1731 (C═O), 1599 (C═N). ^{1}H NMR: (δ ppm, $CDCl_3$, 300 MHz): 1.76–2.09 (m, 15H, adamantan), 7.58–7.69 (m, 2H, Ar—H), 7.85 (dd, J_1 = 7,8 Hz, J_2 = 1,8 Hz, 1H, Ar—H), 8.25 (s, 1H, CH═). Anal. Calcd. for $C_{21}H_{20}BrN_3OS$ (MW 442): 57.02, H: 4.56, N: 9.50%. Found: C: 57.10; H: 4.53, N: 9.56%.

4.5. Biological evaluation

4.5.1. Antibacterial activity

The following Gram-negative bacteria were used: *E. coli* (ATCC 35210), *P. aeruginosa* (ATCC 27853), *S. typhimurium* (ATCC 13311), *E. cloacae* (human isolate) and Gram-positive bacteria: *B. cereus* (clinical isolate), *M. flavus* (ATCC 10240), *L. monocytogenes* (NCTC 7973) and *S. aureus* (ATCC 6538). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research 'Siniša Stanković', Belgrade, Serbia. The antibacterial assay was carried out by a microdilution method (Hanel and Raether, 1988; Daouk et al., 1995) in order to determine the antibacterial activity of compounds tested against the human pathogenic bacteria. The bacterial suspensions were adjusted with sterile saline to a concentration of $1.0 * 10^5$ CFU/mL. The inocula were prepared daily and stored at +4 °C until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum. All experiments were performed in duplicate and repeated three times.

4.5.2. Microdilution test

The minimum inhibitory and bactericidal concentrations (MICs and MBCs) were determined using 96-well microtitre plates. The bacterial suspension was adjusted with sterile saline to a concentration of $1.0 * 10^5$ CFU/mL. Compounds to be investigated were dissolved in 5% DMSO solution containing 0.1% Tween 80 (v/v) (1 mg/mL) and added in broth LB medium (100 µl) with bacterial inoculum ($1.0 * 10^4$ CFU per well) to achieve the wanted concentrations. The microplates were incubated at Rotary shaker (160 rpm) for 24 h at 37 °C. The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The MBCs were determined by serial sub-cultivation of 2 µL into microtitre plates containing 100 µL of broth per well and further incubation for 24 h. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by Microplate manager 4.0 (Bio-Rad Laboratories) and compared with a blank and the positive control. Streptomycin (Sigma P 7794) and Ampicillin (Panfarma, Belgrade, Serbia) were used as a positive control (1 mg/mL in sterile physiological saline). Solution of 5% DMSO was used as a negative control. All experiments were performed in duplicate and repeated three times.

4.5.3. Antifungal activity

For the antifungal bioassays, eight fungi were used: *A. niger* (ATCC 6275), *A. ochraceus* (ATCC 12066), *A. fumigatus* (human isolate), *A. versicolor* (ATCC 11730), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112), *T. viride* (IAM 5061) and *C. albicans* (human isolate). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research 'Siniša Stanković', Belgrade, Serbia. The micromycetes were maintained on malt agar and the cultures stored

at 4 °C and sub-cultured once a month (Booth, 1971). In order to investigate the antifungal activity of the compounds, a modified microdilution technique was used (Daouk et al., 1995; Booth et al., 1971; Espinel-Ingroff, 2001). The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately $1.0 * 10^5$ in a final volume of 100 µL per well. The inocula were stored at 4 °C for further use. Dilutions of the inocula were cultured on solid malt agar to verify the absence of contamination and to check the validity of the inoculum. Minimum inhibitory concentration (MIC) determinations were performed by a serial dilution technique using 96-well microtiter plates. The compounds investigated were dissolved in 5% DMSO solution containing 0.1% Tween 80 (v/v) (1 mg/mL) and added in broth Malt medium 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 a 2 µL of tested compounds 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. Solution of 5% DMSO was used as a negative control, commercial fungicides, bifonazole (Srbolek, Belgrade, Serbia) and ketoconazole (Zorkapharma, Šabac, Serbia), were used as positive controls (1–3500 µg/mL). All experiments were performed in duplicate and repeated three times.

4.6. Prediction of molecular properties

A next step was to proceed with the prediction of physically significant descriptors and pharmaceutically relevant properties of the synthesized molecules. The QikProp (Maestro 9.8) as a fast and accurate prediction software was used.

4.7. Multivariate data analysis

The aforementioned molecular properties in relation to their antifungal and antibacterial activities constituted the dataset that was further subjected to multivariate data analysis. In particular the SIMCA-P version 13.0 software (Umetrics, Umeå, Sweden) was utilized in order to implement Principal Component Analysis (PCA).

A PCA model estimates the systematic variation in a data matrix by a low dimensional model plane. The unsupervised PCA pattern recognition method manages to extract the dominant patterns in the data matrix in terms of a complementary set of scores and loading plots, thus enabling a reduction of dimensionality, a data exploration finding relationship between objects, an estimation of the correlation structure of the variables and investigation on the necessary components (a linear combination of original features) to explain the greater part of variance with a minimum loss of information (Trygg et al., 2007).

The PCA model were UV scaled, extracted at a confidence level of 95% and any observations at $<5\%$ were considered to be outliers.

Loading plots were extracted to reveal the variables that bear class discriminating power.

The quality of models was described by the goodness-of-fit R^2 ($0 \leqslant R^2 \leqslant 1$) and the predictive ability Q^2 ($0 \leqslant Q^2 \leqslant 1$) values. The R^2 explains the variation, thus constitutes a quantitative measure of how well the data of the training set is mathematically reproduced. The overall predictive ability of the model is assessed by the cumulative Q^2 representing the fraction of the variation of Y that can be predicted by the model and was extracted according to the internal cross validation default method of SIMCA-P software (Eriksson et al., 2006).

In particular, all models demonstrated high statistical values ($R^2 > 0.7$ and $Q^2 \geqslant 0.6$), the difference between the goodness-of-fit and the predictive ability remained always lower than 0.2 ($R^2X(cum) - Q^2(cum) < 0.2$) and the goodness-of-fit never equalled to one ($R^2X(cum) \neq 1$). Therefore, since the extracted models abide by these rules then their robustness and predictive response are enhanced (Geladi et al., 1989).

5. Material and methods for generation of pharmacophore

In order to perceive the important functional groups having contributory role for fungal inhibition in *A. fumigatus* and *C. albicans* common feature pharmacophore model (CFPM) was developed using the synthesized compounds by HipHop module in Discovery Studio (DS2.0) (Bhunia et al., 2015).

5.1. Selection of training set

In order to generate pharmacophore models six compounds **8, 16, 18, 19, 23** and **25** having promising inhibitory activity ($IC_{50} < 35$ µM) toward *Aspergillus fumigatus* and *C. albicans* were selected from the dataset of 25 molecules and 18 molecules respectively and rest of the molecules were kept for extending the pharmacophore model. As observed, the dataset of synthesized molecules has variation in functionality regarding the substituent at the phenyl ring, hence the criteria for training set selection was based on the active molecules with good diversity in functionality at this position.

5.2. Generation of pharmacophore model

The selected training set was utilized to develop CFPM for both *A. fumigatus* and *C. albicans* for detection of important chemical functionalities guiding activity. The HipHop module in DS2.0 identifies common chemical feature pattern by over laying molecules in the training set. The chemical functions namely hydrogen bonding acceptor (HBA), ring aromatic (RA), and hydrophobic aliphatic group (HAl), was selected based on optimization procedure and the chemical features present in the training set. Variations in the parameters related to Maximum Omitted Features, Misses, and Complete Misses were done due to the possibility of presence and absence of chemical features in some compounds of the training set. In this context, the "principal number" was set to 2 that ensure that all chemical features in the compounds are considered during generation of hypothesis space. The "maximum omitting features" was set to 0 that force the mapping of chemical features with the pharmacophore features. All the parameters were kept default for the generation CFPM in both cases (*A. fumigatus* and *C. albicans*). In hypothesis generation run

top 10 possible pharmacophore hypotheses were sorted on the basis of ranking score.

5.3. Conformation generation

The structures in the dataset were built using the 2D editor ISIS draw 2.5 and imported to (DS2.0) window for the generation of 3D structures. The conformational search for each molecule was next performed utilizing the best quality conformational search option in DS2.0 keeping the energy threshold constraint to 20 kcal mol^{-1} above the global energy minimum. To ensure proper conformation sampling, a maximum of 255 conformations in BEST mode were generated for each structure.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.arabjc.2016.06.007.

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