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New *N*-(2-phenyl-4-oxo-1,3-thiazolidin-3-yl)-1,2-benzothiazole-3-carboxamides and acetamides as antimicrobial agents†

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A series of 21 novel *N*-[2-phenyl-4-oxo-1,3-thiazolidin-3-yl]-1,2-benzothiazole-3-carboxamides/acetamides (**4a–4p**) as well as a series of *N'*-(halophenylmethylidene)-1,2-benzothiazole-3-acetohydrazides (**3h–3p**) have been synthesized and evaluated for their antimicrobial activity against eight bacterial and eight fungal species, among them plant, animal and human pathogens and food contaminating species. All compounds appeared to be potent and the best activity was exhibited by compound **4d** with MIC in the range of 10.7–21.4 $\mu\text{mol mL}^{-1} \times 10^{-2}$ and MBC of 21.4–40.2 $\mu\text{mol mL}^{-1} \times 10^{-2}$. The best antifungal activity was observed for compounds **4p** and **3h**. Elucidation of the relationship between the antimicrobial activity and molecular properties of the synthesized compounds was also performed. Synthetic intermediates were also tested with several exhibiting good antimicrobial activities. Docking studies for some compounds were performed.

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1. Introduction

Antimicrobial resistance is a growing problem worldwide, requiring a concerted international approach. In fact, since their discovery during the 20th century, antimicrobial agents have substantially reduced the threat posed by infectious diseases.¹

The use of these “wonder drugs”, combined with improvements in sanitation, housing, and nutrition as well as the advent of widespread immunization programmes, has led to a dramatic drop in deaths from diseases that were previously widespread, untreatable, and frequently fatal.

These gains are now seriously jeopardized by another recent development: the emergence and spread of microbes that are resistant to cheap and effective first-choice or “first-line” drugs.

The bacterial infections that contribute most to human disease are also those in which emerging and existent micro-

bial resistance is most evident. Thus, the development of new antimicrobial agents with different modes of action to avoid cross-resistance is increasingly important.²

Five-membered heterocycles with two or three heteroatoms, such as imidazoles, thiazoles, triazoles and others, are key structural units in many pharmaceutical preparations. Moreover, the potential of 4-thiazolidinone ring systems, as core structures in various synthetic compounds displaying antibacterial and antifungal activity, is well known and was confirmed by our recent findings.^{3–9}

In addition, benzo-fused 1,2-thiazole derivatives have been investigated over a period of time by our group, discovering many compounds possessing interesting biological properties including antimicrobial activity.^{10–16}

We previously designed and synthesized some 2-benzothiazole/benzisothiazole 5-benzylidene-4-thiazolidinones as antimicrobial agents. It was found that, in most cases, benzisothiazole derivatives appeared to be less active than 2-benzothiazole-5-benzylidene-4-thiazolidinones. Furthermore, the position of the substituent exerts, in general, a certain effect among the isomeric chloro-substituted compounds.¹⁵ It was observed that the introduction of the bulky electron-withdrawing chloro-group led to enhanced antimicrobial activity.

Our aim now, supported by our previous findings, was to address the synthesis of a new class of *N*-(2-phenyl-4-oxo-1,3-thiazolidin-3-yl)-1,2-benzothiazole-3-carboxamides and acetamides derived from *N'*-phenylmethylidene-1,2-benzothiazole-

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3-carbohydrazides and acetohydrazides, respectively, and their evaluation as antibacterial and antifungal agents.

The novel structures of six of the intermediate hydrazides encouraged us to also test their antimicrobial properties.

2. Results and discussion

2.1. Chemistry

The target compounds *N*-[2-phenyl-4-oxo-1,3-thiazolidin-3-yl]-1,2-benzothiazole-3-carboxamides/acetamides (**4a–p**), mostly halophenyl substituted, were synthesized using a step-wise reaction protocol (Scheme 1), starting from 1,2-benzothiazole-3-carbohydrazide **1** or 1,2-benzothiazole-3-acetohydrazide **2**, synthesized using procedures reported by us previously.¹⁰

Condensation of the amino group of carbohydrazide **1** and its higher homologue **2** with the carbonyl group of commercially available aldehydes afforded the *N*-(phenylmethylidene)-1,2-benzothiazole-3-carbo/acetohydrazides (**3a–q**) in good yields. The reaction of the hydrazides **3a–p** with sulfanylacetic acid in refluxing toluene afforded the final compounds **4a–p**. The structure and purity of the novel compounds **3h–j**, **3o–q** and **4a–p** were confirmed by ¹H NMR, MS and IR spectroscopy and elemental analyses. The characterization of previously reported hydrazides **3a–g** and **3k–n** was consistent with the literature data.⁹

2.2. Biological evaluations

2.2.1. Antibacterial activity. The results of antimicrobial activity of synthetic compounds (**3h**, **i**, **j**, **o**, and **p** and **4a–p**) and standard antibiotics/antimycotics against a panel of selected Gram-positive and Gram-negative bacteria and fungi

are presented in Tables 1 and 2, respectively. The minimal inhibitory and minimal bactericidal/fungicidal concentrations were determined using a microdilution method.

The synthetic compounds showed antibacterial activity against all tested bacteria but on different levels. The minimal inhibitory concentrations (MIC) ranged between 10.2–78.9 $\mu\text{mol mL}^{-1} \times 10^{-2}$, while the minimal bactericidal concentrations (MBC) were 14.7–115.2 $\mu\text{mol mL}^{-1} \times 10^{-2}$. The antibacterial potential can be presented as following: **4d** > **4h** > **4f** > **4m** > **4c** > **4a** > **4p** > **4i** > **4b** > **4k** > **4o** > **4l** > **4e** > **4n** > **4j** > **4g**. The best antibacterial activity was obtained for compound **4d**, which exhibited a 10.7–21.4 $\mu\text{mol mL}^{-1} \times 10^{-2}$ range of MIC and a 21.4–40.2 $\mu\text{mol mL}^{-1} \times 10^{-2}$ range of MBC. The lowest antibacterial activity among all the compounds tested was obtained for **4g** (MIC 20.5–78.9 $\mu\text{mol mL}^{-1} \times 10^{-2}$ and MBC 38.4–115.2 $\mu\text{mol mL}^{-1} \times 10^{-2}$). The most sensitive bacterial species among Gram-positive bacteria was *Staphylococcus aureus*, while the most resistant species was *Listeria monocytogenes*. For Gram-negative bacteria, the most sensitive was *Escherichia coli*, while *Salmonella typhimurium* was the most resistant. The standard drugs used as positive controls, streptomycin and ampicillin, were also active against all the bacteria.

The MIC range for streptomycin was 4.3–25.8 $\mu\text{mol mL}^{-1} \times 10^{-2}$ and the range of MBC was 8.6–51.6 $\mu\text{mol mL}^{-1} \times 10^{-2}$, while ampicillin showed a slightly lower antibacterial potential with a range of MIC 24.8–74.4 $\mu\text{mol mL}^{-1} \times 10^{-2}$ and MBC 37.2–124.0 $\mu\text{mol mL}^{-1} \times 10^{-2}$. All the tested compounds possessed strong antibacterial activity and showed similar or higher effects than ampicillin and, in many cases, were better than streptomycin. It should be noted that all compounds showed very good antibacterial effects against *Pseudomonas aeruginosa*, being much better than ampicillin.

Furthermore, some novel intermediate compounds (**3h**, **i**, **j**, **o** and **p**) were also evaluated for their antimicrobial activity.

The results of antimicrobial activity of intermediates **3h–j** and **3o** and **p** and standard antibiotics/antimycotics against a panel of selected Gram-positive and Gram-negative bacteria and fungi are presented in Table 1. The minimal inhibitory and minimal bactericidal/fungicidal concentrations were determined using a microdilution method.

The synthetic compounds showed antibacterial activity against all the tested bacteria but on different levels to compounds **4a–p**. Minimal inhibitory concentrations (MIC) ranged between 2.9–36.2 $\mu\text{mol mL}^{-1} \times 10^{-2}$, while minimal bactericidal concentrations (MBC) were between 6.3–96.4 $\mu\text{mol mL}^{-1} \times 10^{-2}$. The antibacterial potential follows the order: **3o** > **3j** > **3h** > **3i** > **3p**.

The best antibacterial activity was obtained for compound **3o**, with a MIC range of 2.9–11.6 $\mu\text{mol mL}^{-1} \times 10^{-2}$ and an MBC at 23.26 $\mu\text{mol mL}^{-1} \times 10^{-2}$. The lowest antibacterial activity among all compounds tested in this study was obtained for **3p** (MIC 24.1–36.2 $\mu\text{mol mL}^{-1} \times 10^{-2}$ and MBC 48.2–96.4 $\mu\text{mol mL}^{-1} \times 10^{-2}$). The most sensitive bacterial species were *Staphylococcus aureus* and *Salmonella typhimurium*, while the most resistant species were *Listeria monocytogenes* and *Pseudomonas*

Comp/d	n	R	Comp/ds	n	R
a	0	H	j	0	2,6-Cl ₂
b	0	2-F	k	1	H
c	0	3-F	l	1	2-Cl
d	0	4-F	m	1	3-Cl
e	0	2-Cl	n	1	4-Cl
f	0	3-Cl	o	1	2,6-F ₂
g	0	4-Cl	p	1	2-F,6-Cl
h	0	2,6-F ₂	q	1	2,6-Cl ₂
i	0	2-F,6-Cl			

Scheme 1 Synthesis of title compounds. Reagents and conditions: (a) $\text{R-C}_6\text{H}_4\text{CHO}$, CH_3COOH , EtOH, reflux, 3 h; (b) HSCH_2COOH , $\text{CH}_3\text{C}_6\text{H}_5$, reflux, 48 h.

Table 1 Antibacterial activity of compounds **3h**, **i**, **j**, **o**, **p** and **4a–4p** (MIC and MBC in $\mu\text{mol mL}^{-1} \times 10^{-2}$)

Compounds		<i>B.c.</i>	<i>M.f.</i>	<i>S.a.</i>	<i>L.m.</i>	<i>E.c.</i>	<i>En.cl.</i>	<i>P.a.</i>	<i>S.t.</i>
3h	MIC	12.6 $\pm$ 0.3	25.2 $\pm$ 2.1	12.6 $\pm$ 0.6	25.2 $\pm$ 0.8	25.2 $\pm$ 0.6	6.3 $\pm$ 0.1	25.2 $\pm$ 2.1	3.15 $\pm$ 0.1
	MBC	25.2 $\pm$ 1.2	50.4 $\pm$ 1.2	25.2 $\pm$ 1.2	50.4 $\pm$ 1.2	50.4 $\pm$ 1.2	25.2 $\pm$ 0.6	50.4 $\pm$ 2.1	6.3 $\pm$ 0.2
3i	MIC	12.0 $\pm$ 0.6	12.0 $\pm$ 0.8	6.0 $\pm$ 0.0	24.0 $\pm$ 0.8	12.0 $\pm$ 0.0	6.0 $\pm$ 0.0	24.0 $\pm$ 0.8	6.0 $\pm$ 0.0
	MBC	24.0 $\pm$ 2.1	24.0 $\pm$ 1.2	12.0 $\pm$ 0.2	48.0 $\pm$ 0.6	24.0 $\pm$ 0.6	12.0 $\pm$ 0.1	48.0 $\pm$ 2.1	12.0 $\pm$ 0.6
3j	MIC	11.6 $\pm$ 0.6	11.6 $\pm$ 0.3	5.8 $\pm$ 0.5	23.2 $\pm$ 0.6	11.6 $\pm$ 0.8	11.6 $\pm$ 0.3	23.2 $\pm$ 0.2	11.6 $\pm$ 1.8
	MBC	23.2 $\pm$ 0.8	23.2 $\pm$ 1.2	17.6 $\pm$ 0.8	46.4 $\pm$ 1.2	23.2 $\pm$ 0.3	23.2 $\pm$ 0.8	46.4 $\pm$ 1.2	23.2 $\pm$ 0.6
3o	MIC	11.6 $\pm$ 0.8	11.6 $\pm$ 0.6	2.9 $\pm$ 0.1	11.6 $\pm$ 0.6	11.6 $\pm$ 0.5	11.6 $\pm$ 0.1	11.6 $\pm$ 0.6	11.6 $\pm$ 0.5
	MBC	23.2 $\pm$ 0.0	23.2 $\pm$ 0.3	23.2 $\pm$ 0.3	23.2 $\pm$ 0.8	23.2 $\pm$ 0.3	23.2 $\pm$ 0.3	23.2 $\pm$ 0.3	23.2 $\pm$ 0.8
3p	MIC	24.1 $\pm$ 1.2	36.2 $\pm$ 0.2	24.1 $\pm$ 0.8	48.2 $\pm$ 0.8	36.2 $\pm$ 0.2	36.2 $\pm$ 0.2	36.2 $\pm$ 0.2	24.1 $\pm$ 0.3
	MBC	48.2 $\pm$ 2.8	48.2 $\pm$ 1.2	48.2 $\pm$ 1.2	96.4 $\pm$ 1.2	48.2 $\pm$ 1.2	48.2 $\pm$ 0.6	48.2 $\pm$ 1.2	48.2 $\pm$ 1.2
4a	MIC	22.5 $\pm$ 0.9	22.5 $\pm$ 0.6	22.5 $\pm$ 0.3	22.5 $\pm$ 0.3	11.2 $\pm$ 0.3	11.2 $\pm$ 0.2	22.5 $\pm$ 0.6	22.5 $\pm$ 1.0
	MBC	42.2 $\pm$ 0.6	42.2 $\pm$ 0.9	42.2 $\pm$ 0.6	42.2 $\pm$ 0.3	22.5 $\pm$ 0.2	22.5 $\pm$ 0.8	42.2 $\pm$ 0.3	42.2 $\pm$ 0.6
4b	MIC	21.4 $\pm$ 0.2	29.5 $\pm$ 0.6	21.4 $\pm$ 0.6	21.4 $\pm$ 0.3	21.4 $\pm$ 0.2	21.4 $\pm$ 0.9	21.4 $\pm$ 0.6	21.4 $\pm$ 0.6
	MBC	40.2 $\pm$ 0.9	40.2 $\pm$ 1.2	40.2 $\pm$ 1.5	40.2 $\pm$ 0.9	40.2 $\pm$ 0.9	40.2 $\pm$ 0.6	40.2 $\pm$ 1.2	40.2 $\pm$ 1.0
4c	MIC	10.7 $\pm$ 0.6	21.4 $\pm$ 0.9	10.7 $\pm$ 0.3	21.4 $\pm$ 0.6	16.0 $\pm$ 0.3	21.4 $\pm$ 0.9	21.4 $\pm$ 0.9	21.4 $\pm$ 1.0
	MBC	40.2 $\pm$ 1.2	40.2 $\pm$ 1.3	21.4 $\pm$ 0.9	40.2 $\pm$ 0.8	21.4 $\pm$ 0.6	21.4 $\pm$ 0.6	80.4 $\pm$ 2.1	40.2 $\pm$ 1.2
4d	MIC	21.4 $\pm$ 0.6	21.4 $\pm$ 0.3	10.7 $\pm$ 0.1	21.4 $\pm$ 0.9	10.7 $\pm$ 0.6	10.7 $\pm$ 0.00	10.7 $\pm$ 0.3	10.7 $\pm$ 0.2
	MBC	40.2 $\pm$ 1.2	40.2 $\pm$ 0.9	21.4 $\pm$ 0.3	40.2 $\pm$ 0.6	21.4 $\pm$ 0.6	21.4 $\pm$ 0.3	21.4 $\pm$ 0.9	21.4 $\pm$ 0.6
4e	MIC	15.4 $\pm$ 0.6	38.4 $\pm$ 0.9	10.2 $\pm$ 0.6	58.9 $\pm$ 1.2	28.2 $\pm$ 0.3	20.5 $\pm$ 0.9	28.2 $\pm$ 0.3	20.5 $\pm$ 0.6
	MBC	38.4 $\pm$ 0.6	76.8 $\pm$ 1.0	38.4 $\pm$ 0.3	76.8 $\pm$ 0.6	38.4 $\pm$ 1.2	38.4 $\pm$ 0.6	38.4 $\pm$ 0.6	38.4 $\pm$ 0.3
4f	MIC	10.2 $\pm$ 0.6	20.5 $\pm$ 0.3	10.2 $\pm$ 0.8	2.05 $\pm$ 0.06	10.2 $\pm$ 1.2	20.5 $\pm$ 0.9	20.5 $\pm$ 0.6	20.5 $\pm$ 0.3
	MBC	38.4 $\pm$ 1.2	38.4 $\pm$ 1.2	20.5 $\pm$ 0.6	3.84 $\pm$ 0.1	20.5 $\pm$ 0.6	38.4 $\pm$ 0.9	38.4 $\pm$ 1.0	38.4 $\pm$ 1.2
4g	MIC	20.5 $\pm$ 0.6	38.4 $\pm$ 0.9	20.5 $\pm$ 1.2	58.9 $\pm$ 2.1	58.9 $\pm$ 2.0	78.9 $\pm$ 1.8	20.5 $\pm$ 0.8	76.8 $\pm$ 1.6
	MBC	38.4 $\pm$ 1.3	76.8 $\pm$ 1.0	38.4 $\pm$ 0.9	76.8 $\pm$ 1.2	76.8 $\pm$ 2.1	115.2 $\pm$ 3.2	76.8 $\pm$ 2.1	115.2 $\pm$ 3.5
4h	MIC	10.2 $\pm$ 0.8	10.2 $\pm$ 0.6	10.2 $\pm$ 0.3	20.4 $\pm$ 0.6	10.2 $\pm$ 0.6	10.2 $\pm$ 0.3	20.4 $\pm$ 0.8	20.4 $\pm$ 0.6
	MBC	20.4 $\pm$ 0.8	20.4 $\pm$ 0.6	20.4 $\pm$ 0.6	40.8 $\pm$ 1.2	20.4 $\pm$ 1.0	20.4 $\pm$ 0.8	40.8 $\pm$ 1.2	40.8 $\pm$ 1.0
4i	MIC	19.6 $\pm$ 0.3	27.0 $\pm$ 0.6	19.6 $\pm$ 1.2	27.0 $\pm$ 0.9	27.0 $\pm$ 0.6	36.8 $\pm$ 1.0	19.6 $\pm$ 0.6	19.6 $\pm$ 0.6
	MBC	36.8 $\pm$ 1.2	36.8 $\pm$ 0.6	36.8 $\pm$ 0.9	36.8 $\pm$ 2.1	36.8 $\pm$ 2.1	73.5 $\pm$ 2.5	36.8 $\pm$ 0.9	36.8 $\pm$ 0.6
4j	MIC	39.5 $\pm$ 0.9	54.3 $\pm$ 1.2	18.9 $\pm$ 0.6	39.5 $\pm$ 1.0	54.3 $\pm$ 1.0	54.3 $\pm$ 0.9	18.9 $\pm$ 1.0	39.5 $\pm$ 1.2
	MBC	79.0 $\pm$ 2.2	79.0 $\pm$ 2.0	39.5 $\pm$ 1.0	79.0 $\pm$ 2.0	79.0 $\pm$ 1.0	79.0 $\pm$ 0.9	39.5 $\pm$ 1.2	79.0 $\pm$ 1.0
4k	MIC	21.6 $\pm$ 0.6	29.7 $\pm$ 0.6	21.6 $\pm$ 0.9	21.6 $\pm$ 1.0	21.6 $\pm$ 1.0	10.8 $\pm$ 0.6	21.6 $\pm$ 0.6	21.6 $\pm$ 0.6
	MBC	40.5 $\pm$ 1.0	40.5 $\pm$ 2.0	40.5 $\pm$ 2.1	40.5 $\pm$ 1.6	40.5 $\pm$ 1.8	21.4 $\pm$ 0.6	40.5 $\pm$ 2.1	40.5 $\pm$ 2.0
4l	MIC	19.8 $\pm$ 0.9	27.3 $\pm$ 0.6	19.8 $\pm$ 0.6	37.2 $\pm$ 0.3	19.8 $\pm$ 0.3	27.3 $\pm$ 0.3	37.2 $\pm$ 0.6	19.8 $\pm$ 0.00
	MBC	37.2 $\pm$ 0.6	37.2 $\pm$ 0.6	37.2 $\pm$ 0.3	74.4 $\pm$ 1.2	37.2 $\pm$ 1.2	37.2 $\pm$ 0.9	74.4 $\pm$ 2.0	37.2 $\pm$ 0.6
4m	MIC	19.8 $\pm$ 0.6	19.8 $\pm$ 0.3	10.0 $\pm$ 0.2	19.8 $\pm$ 0.6	19.8 $\pm$ 0.3	19.8 $\pm$ 0.6	19.8 $\pm$ 1.0	10.0 $\pm$ 0.0
	MBC	37.2 $\pm$ 1.2	37.2 $\pm$ 0.9	37.2 $\pm$ 0.6	37.2 $\pm$ 0.3	37.2 $\pm$ 0.3	37.2 $\pm$ 0.6	37.2 $\pm$ 0.6	37.2 $\pm$ 0.8
4n	MIC	10.0 $\pm$ 0.6	27.3 $\pm$ 1.0	19.8 $\pm$ 0.6	74.4 $\pm$ 1.2	27.3 $\pm$ 1.0	57.0 $\pm$ 2.1	37.2 $\pm$ 0.9	37.2 $\pm$ 0.9
	MBC	19.8 $\pm$ 0.6	37.2 $\pm$ 0.6	37.2 $\pm$ 0.9	111.6 $\pm$ 3.1	37.2 $\pm$ 1.0	74.4 $\pm$ 2.0	74.4 $\pm$ 1.5	74.4 $\pm$ 1.2
4o	MIC	19.8 $\pm$ 0.6	27.2 $\pm$ 0.3	19.8 $\pm$ 0.3	54.3 $\pm$ 1.2	27.2 $\pm$ 1.2	19.8 $\pm$ 0.6	27.2 $\pm$ 0.6	19.8 $\pm$ 0.3
	MBC	37.0 $\pm$ 1.2	37.0 $\pm$ 0.6	37.0 $\pm$ 0.3	74.0 $\pm$ 1.2	37.0 $\pm$ 1.2	37.0 $\pm$ 1.0	37.0 $\pm$ 0.6	37.0 $\pm$ 0.3
4p	MIC	14.7 $\pm$ 0.6	27.0 $\pm$ 0.6	14.7 $\pm$ 0.3	36.7 $\pm$ 0.3	27.0 $\pm$ 0.5	14.7 $\pm$ 0.3	27.0 $\pm$ 0.3	19.6 $\pm$ 0.6
	MBC	36.7 $\pm$ 1.2	36.7 $\pm$ 0.9	19.6 $\pm$ 0.3	73.4 $\pm$ 1.2	36.7 $\pm$ 0.3	19.6 $\pm$ 0.3	36.7 $\pm$ 0.3	36.7 $\pm$ 0.3
Streptomycin	MIC	4.3 $\pm$ 0.06	8.6 $\pm$ 0.03	17.2 $\pm$ 0.1	25.8 $\pm$ 0.3	17.2 $\pm$ 0.6	4.3 $\pm$ 0.03	17.2 $\pm$ 0.6	17.2 $\pm$ 0.3
	MBC	8.6 $\pm$ 0.03	17.2 $\pm$ 0.6	34.4 $\pm$ 0.3	51.6 $\pm$ 0.8	34.4 $\pm$ 0.9	8.6 $\pm$ 0.6	34.4 $\pm$ 0.3	34.4 $\pm$ 0.5
Ampicillin	MIC	24.8 $\pm$ 0.2	24.8 $\pm$ 0.3	24.8 $\pm$ 0.1	37.2 $\pm$ 0.3	37.2 $\pm$ 0.3	24.8 $\pm$ 0.2	74.4 $\pm$ 0.8	24.8 $\pm$ 0.6
	MBC	37.2 $\pm$ 0.2	37.2 $\pm$ 0.1	37.2 $\pm$ 0.3	74.4 $\pm$ 0.8	49.2 $\pm$ 0.6	37.2 $\pm$ 0.3	124.0 $\pm$ 1.2	49.2 $\pm$ 0.8

B.c.: *Bacillus cereus* (clinical isolate), *M.f.*: *Micrococcus flavus* (ATCC 10240), *S.a.*: *Staphylococcus aureus* (ATCC 6538), *L.m.*: *Listeria monocytogenes* (NCTC 7973), *E. coli*: *Escherichia coli* (ATCC 35210), *En.Cl.*: *Enterobacter cloacae* (human isolate), *P.a.*: *Pseudomonas aeruginosa* (ATCC 27853), *S.t.*: *Salmonella typhimurium* (ATCC 13311).

aeruginosa. The standard drugs used as positive controls, streptomycin and ampicillin, were also active against all the bacteria.

All the tested compounds possessed strong antibacterial activity and showed similar or better effects compared to streptomycin and higher activities than ampicillin.

Concerning compounds **3**, analyses of structure–activity relationships revealed that antibacterial activity depends upon the nature of each aromatic substituent and also on the number of methylene groups. Thus, among compounds with $n = 0$ it seems that the most favourable substitution for antibacterial activity is the 2,6-di-chloro-(**3j**), followed by the di-fluoro-(**3h**) and the less favorable appeared to be 2-chloro-6-

fluoro-(**3i**) substitution. On the other hand, for compounds with $n = 1$ the best activity was observed for the 2-chloro-6-fluoro substituted derivative.

Referring to compounds **4a–p**, analyses of structure–activity relationships revealed that antibacterial activity depends, as in the case of compounds **3**, not only on the nature of the substituents but also on their position at the aromatic ring. Thus, for chloro-derivatives ($n = 0$) position **3** is the most favourable and the relative activity of these compounds follows $3\text{-Cl} > 2\text{-Cl} > 4\text{-Cl}$. This order was also observed for chloro-derivatives with $n = 1$, showing that the addition of one more methylene group

Table 2 Antifungal activity of compounds **3h–3p** and **4a–4p** (MIC and MFC in $\mu\text{mol mL}^{-1} \times 10^{-2}$)

Compounds		<i>A.fum.</i>	<i>A.v.</i>	<i>A.o.</i>	<i>A.n.</i>	<i>T.v.</i>	<i>P.f.</i>	<i>P.o.</i>	<i>C.a.</i>
3h	MIC	1.58 ± 0.3	1.58 ± 0.3	1.58 ± 0.1	25.2 ± 0.1	12.6 ± 1.2	3.15 ± 0.6	12.6 ± 0.8	25.2 ± 0.2
	MFC	25.2 ± 0.3	12.6 ± 0.2	12.6 ± 0.1	50.4 ± 0.6	25.2 ± 0.3	12.6 ± 0.3	25.2 ± 0.2	50.4 ± 1.2
3i	MIC	24.0 ± 0.8	6.0 ± 0.3	6.0 ± 0.6	6.0 ± 1.0	24.0 ± 1.2	3.0 ± 0.3	6.0 ± 0.6	24.0 ± 0.9
	MFC	30.0 ± 2.0	12.0 ± 0.8	12.0 ± 0.6	12.0 ± 0.3	48.0 ± 1.2	6.0 ± 0.6	12.0 ± 0.3	30.0 ± 1.2
3j	MIC	23.2 ± 0.1	46.4 ± 0.0	23.2 ± 1.0	46.4 ± 1.2	11.6 ± 1.2	46.4 ± 2.1	23.2 ± 1.2	23.2 ± 1.0
	MFC	46.4 ± 0.2	58.0 ± 2.2	46.4 ± 1.0	58.0 ± 0.6	46.4 ± 1.2	58.0 ± 2.0	46.4 ± 1.0	46.4 ± 1.2
3o	MIC	48.2 ± 2.1	9.1 ± 0.6	12.1 ± 0.3	12.1 ± 0.6	6.1 ± 0.6	9.1 ± 0.6	18.1 ± 0.6	12.1 ± 0.3
	MFC	96.4 ± 1.6	12.1 ± 0.6	24.1 ± 1.0	24.1 ± 0.8	12.1 ± 0.8	12.1 ± 0.6	24.1 ± 0.8	24.1 ± 0.6
3p	MIC	46.4 ± 1.2	11.6 ± 0.6	11.6 ± 0.8	11.6 ± 1.0	11.6 ± 0.8	11.6 ± 0.6	11.6 ± 0.3	46.4 ± 2.0
	MFC	92.8 ± 3.2	23.2 ± 0.8	23.2 ± 0.6	23.2 ± 1.2	23.2 ± 0.6	23.2 ± 0.6	23.2 ± 0.3	58.0 ± 1.2
4a	MIC	11.2 ± 0.6	22.5 ± 1.0	2.80 ± 0.06	11.2 ± 0.1	5.60 ± 0.06	22.5 ± 0.6	11.2 ± 0.6	25.3 ± 0.5
	MFC	22.5 ± 0.3	42.2 ± 2.1	5.60 ± 0.06	22.5 ± 0.4	11.2 ± 0.6	42.2 ± 1.2	22.5 ± 0.9	42.2 ± 2.0
4b	MIC	10.7 ± 0.9	21.4 ± 0.3	8.00 ± 0.03	10.7 ± 0.6	2.7 ± 0.06	10.7 ± 0.1	5.4 ± 0.03	10.7 ± 0.6
	MFC	21.4 ± 0.1	40.2 ± 1.2	21.4 ± 0.9	21.4 ± 0.1	5.4 ± 0.06	21.4 ± 0.6	10.7 ± 0.9	21.4 ± 1.2
4c	MIC	5.4 ± 0.03	5.4 ± 0.03	29.2 ± 0.9	10.7 ± 0.6	5.4 ± 0.00	10.7 ± 0.6	10.7 ± 0.6	10.7 ± 0.3
	MFC	10.7 ± 0.3	10.7 ± 0.6	40.2 ± 2.3	21.4 ± 1.2	10.7 ± 0.9	21.4 ± 0.3	21.4 ± 0.3	21.4 ± 0.6
4d	MIC	10.7 ± 0.6	40.2 ± 2.1	40.2 ± 2.0	40.2 ± 2.1	40.2 ± 2.0	21.4 ± 0.9	40.2 ± 2.1	8.0 ± 0.1
	MFC	21.4 ± 0.6	80.4 ± 2.1	80.4 ± 2.0	80.4 ± 2.3	80.4 ± 1.2	53.6 ± 1.0	80.4 ± 1.2	10.7 ± 0.6
4e	MIC	10.25 ± 0.1	2.56 ± 0.1	10.2 ± 0.3	10.2 ± 0.4	10.2 ± 0.2	20.5 ± 0.6	38.4 ± 1.0	38.4 ± 0.9
	MFC	20.5 ± 0.6	5.1 ± 0.1	20.5 ± 0.6	20.5 ± 0.6	20.5 ± 0.6	38.4 ± 1.2	76.8 ± 2.1	76.8 ± 2.3
4f	MIC	38.4 ± 0.3	5.1 ± 0.1	10.2 ± 0.1	7.7 ± 0.06	5.1 ± 0.03	10.2 ± 0.9	20.8 ± 0.3	10.2 ± 0.3
	MFC	76.8 ± 1.2	10.2 ± 0.6	20.5 ± 0.3	10.2 ± 0.3	10.2 ± 0.6	20.5 ± 0.5	38.4 ± 1.2	20.5 ± 0.6
4g	MIC	20.5 ± 0.6	10.2 ± 0.3	10.2 ± 0.1	10.2 ± 0.8	2.56 ± 0.03	10.2 ± 0.3	10.2 ± 0.2	10.2 ± 0.1
	MFC	38.4 ± 0.9	20.5 ± 1.0	20.5 ± 1.2	20.5 ± 0.9	5.12 ± 0.06	20.5 ± 0.1	20.5 ± 0.3	20.5 ± 0.3
4h	MIC	40.8 ± 2.1	20.4 ± 0.9	5.1 ± 0.06	20.4 ± 0.3	5.1 ± 0.03	10.2 ± 0.1	10.2 ± 0.3	40.8 ± 1.2
	MFC	51.0 ± 1.2	40.8 ± 0.9	10.2 ± 0.6	40.8 ± 1.0	10.2 ± 0.6	20.4 ± 0.3	51.0 ± 1.0	51.0 ± 1.0
4i	MIC	36.8 ± 1.2	18.4 ± 0.3	36.8 ± 0.6	36.8 ± 0.3	13.8 ± 0.6	36.8 ± 0.3	36.8 ± 0.6	73.5 ± 2.2
	MFC	73.5 ± 2.3	73.5 ± 2.1	73.5 ± 2.0	73.5 ± 1.2	18.4 ± 0.9	73.5 ± 2.2	85.8 ± 2.5	85.8 ± 2.3
4j	MIC	39.5 ± 1.2	18.9 ± 0.9	9.45 ± 0.1	39.5 ± 0.9	13.2 ± 0.6	39.5 ± 1.2	39.5 ± 1.0	39.5 ± 1.0
	MFC	79.0 ± 2.1	70.8 ± 2.5	18.9 ± 0.9	79.0 ± 1.2	18.9 ± 0.3	79.0 ± 1.2	79.0 ± 1.0	79.0 ± 1.2
4k	MIC	10.8 ± 0.00	21.6 ± 0.9	5.4 ± 0.03	16.2 ± 0.1	5.4 ± 0.03	21.6 ± 0.3	16.2 ± 0.6	21.6 ± 0.6
	MFC	40.5 ± 2.1	40.5 ± 2.0	10.8 ± 0.1	21.6 ± 0.3	10.8 ± 0.3	21.6 ± 2.1	21.6 ± 2.0	40.5 ± 2.2
4l	MIC	37.2 ± 0.8	7.4 ± 0.1	10.0 ± 0.00	14.4 ± 0.3	7.4 ± 0.03	10.0 ± 0.00	14.4 ± 0.6	19.8 ± 0.1
	MFC	74.4 ± 2.1	10.0 ± 0.1	19.8 ± 0.3	19.8 ± 0.3	10.0 ± 0.6	19.8 ± 0.8	19.8 ± 0.9	37.2 ± 1.0
4m	MIC	10.0 ± 0.00	10.0 ± 0.1	7.4 ± 0.06	10.0 ± 1.0	5.0 ± 0.3	10.0 ± 0.3	7.4 ± 0.06	10.0 ± 0.8
	MFC	37.2 ± 1.0	19.2 ± 0.6	10.0 ± 1.0	19.2 ± 0.6	10.0 ± 0.3	19.8 ± 0.9	10.0 ± 1.0	19.8 ± 0.3
4n	MIC	37.2 ± 1.0	10.0 ± 0.9	10.0 ± 0.9	19.8 ± 0.6	10.0 ± 0.3	14.4 ± 1.0	10.0 ± 0.00	19.8 ± 0.6
	MFC	74.4 ± 2.5	19.8 ± 0.9	19.8 ± 0.6	37.2 ± 1.0	19.8 ± 0.6	19.8 ± 0.6	19.8 ± 0.3	37.2 ± 0.8
4o	MIC	29.6 ± 0.9	7.4 ± 0.06	7.4 ± 0.06	14.8 ± 0.6	7.4 ± 0.03	7.4 ± 0.00	14.8 ± 0.9	9.9 ± 1.0
	MFC	37.0 ± 1.0	9.9 ± 0.03	9.9 ± 0.03	19.8 ± 0.9	9.9 ± 0.6	9.9 ± 0.3	19.8 ± 0.6	19.8 ± 0.6
4p	MIC	9.8 ± 0.1	4.9 ± 0.03	9.8 ± 0.03	19.6 ± 1.0	3.8 ± 0.06	4.9 ± 0.05	4.9 ± 0.03	4.9 ± 0.03
	MFC	19.6 ± 1.0	9.8 ± 0.6	19.6 ± 1.0	36.7 ± 0.9	4.9 ± 0.03	9.8 ± 0.1	9.8 ± 0.03	9.8 ± 0.06
Ketoconazole	MIC	38.0 ± 0.1	38.0 ± 0.3	28.0 ± 0.2	38.0 ± 0.6	190.0 ± 4.5	38.0 ± 1.2	190.0 ± 1.6	38.0 ± 0.3
	MFC	95.0 ± 2.1	95.0 ± 2.1	38.0 ± 0.6	95.0 ± 1.0	285.0 ± 2.5	95.0 ± 1.8	285.0 ± 3.2	56.0 ± 2.0
Bifonazole	MIC	48.0 ± 1.0	32.0 ± 0.9	48.0 ± 1.0	48.0 ± 0.9	64.0 ± 1.2	64.0 ± 1.0	64.0 ± 1.0	32.0 ± 0.6
	MFC	64.0 ± 1.0	64.0 ± 0.6	80.0 ± 0.9	64.0 ± 0.9	80.0 ± 1.0	80.0 ± 2.1	80.5 ± 2.0	64.0 ± 2.5

A.fum.: *Aspergillus fumigatus* (plant isolate), *A.v.*: *Aspergillus versicolor* (ATCC 11730), *A.o.*: *Aspergillus ochraceus* (ATCC 12066), *A.n.*: *Aspergillus niger* (ATCC 6275), *T.v.*: *Trichoderma viride* (IAM 5061), *P.f.*: *Penicillium funiculosum* (ATCC 36839), *P.o.*: *Penicillium ochrochloron* (ATCC 9112), *C.a.*: *Candida albicans* (human isolate).

between the benzothiazole and C=O group does not affect the activity.

On the other hand, with fluoro-derivatives the most active derivative appeared to be 4-F, followed by 3-F and then 2-F. In the case of double halogen substitution with $n = 0$, the order of activity was as follows: 2,6-di-F > 2-Cl, 6-F > 2,6-di-Cl.

The 2,6-di-fluoro-substitution results in better activity compared to 2-fluoro-6-chloro-substitution. Almost the same was observed when $n = 1$. In general, the most active compounds appeared to be fluoro-derivatives.

It should be noted that the order of activity for intermediates **3h**, **3i**, **3j**, **3o** and **3p** is the opposite of that of the title

compounds where the more preferable substitution was 2,6-difluoro- and the less the 2,6-dichloro-derivative.

2.2.2. Antifungal activity. The antifungal activities of compounds **3h**, **i**, **j**, **o**, and **p** and **4a–p** are presented in Table 2. All the compounds showed good antifungal activities in the MIC range of 1.58–48.2 $\mu\text{mol mL}^{-1} \times 10^{-2}$ and in the MFC range of 6.0–96.4 $\mu\text{mol mL}^{-1} \times 10^{-2}$. The antifungal potential can be presented as follows: **4p** > **4m** > **4o** > **4c** > **4b** > **4g** > **4f** > **4a** > **4k** > **4l** > **4n** > **4e** > **4h** > **4d** > **4j** > **4i**. Compound **4p** exhibited the best fungistatic activity at 3.8–9.8 $\mu\text{mol mL}^{-1} \times 10^{-2}$, and fungicidal activity was achieved at 4.9–36.7 $\mu\text{mol mL}^{-1} \times 10^{-2}$. The lowest

antifungal effect was observed for compound **4i** with MIC $18.4\text{--}73.5 \mu\text{mol mL}^{-1} \times 10^{-2}$ and MFC $18.4\text{--}85.8 \mu\text{mol mL}^{-1} \times 10^{-2}$. The majority of compounds showed the best activity against *T. viride*, while the most resistant was *A. fumigatus*.

The synthetic fungicide bifonazole possessed an inhibitory activity of $48.0\text{--}64.0 \mu\text{mol mL}^{-1} \times 10^{-2}$ and a fungicidal activity of $64.0\text{--}80.0 \mu\text{mol mL}^{-1} \times 10^{-2}$, while ketoconazole showed a lower range of MIC of $38.0\text{--}475.0 \mu\text{mol mL}^{-1} \times 10^{-2}$ and MFC of $95.0\text{--}570.0 \mu\text{mol mL}^{-1} \times 10^{-2}$ (Table 2). However, all the compounds tested showed much stronger antifungal activity than commercial mycotics, particularly ketoconazole (Table 2).

The results of antifungal activity of intermediate compounds **3h**, **3i**, **3j**, **3o** and **3p** are presented in Table 2. All the compounds showed good antifungal activities in the MIC range of $1.58\text{--}48.2 \mu\text{mol mL}^{-1} \times 10^{-2}$ and MFC range of $6.0\text{--}96.4 \mu\text{mol mL}^{-1} \times 10^{-2}$.

The antifungal potential order is: **3h** > **3i** > **3j** > **3o** > **3p**. Compound **3h** exhibited the best fungistatic activity at $1.58\text{--}25.2 \mu\text{mol mL}^{-1} \times 10^{-2}$, and fungicidal activity was achieved at $12.6\text{--}50.4 \mu\text{mol mL}^{-1} \times 10^{-2}$. Compound **3p** exhibited the lowest antifungal effect with MIC of $6.1\text{--}48.2 \mu\text{mol mL}^{-1} \times 10^{-2}$ and MFC of $12.1\text{--}96.4 \mu\text{mol mL}^{-1} \times 10^{-2}$. The majority of the compounds showed high activity against *A. ochraceus*, while the most resistant was *A. fumigatus*.

It should be mentioned that all the compounds tested showed much stronger antifungal activity than commercial mycotics, particularly ketoconazole (MIC of $38.0\text{--}475.0 \mu\text{mol mL}^{-1} \times 10^{-2}$ and MFC of $95.0\text{--}570.0 \mu\text{mol mL}^{-1} \times 10^{-2}$) (Table 2).

Studies of structure–activity relationships confirmed the previous observations that the antifungal activity of the studied compounds depends not only on the nature of the substituent but also on its position on the aromatic ring.

Thus, in the case of compounds **3h**, **i**, **j**, **o**, and **p** it seems that the absence of a methylene group ($n = 0$) has a positive influence on antifungal activity. In these compounds, 2,6-difluoro substitution appeared to be the most favorable. Thus, the only similarity to antibacterial activity was observed for compound **3o**, which showed the lowest antibacterial as well as antifungal activity.

The structure–activity relationship studies of compounds **4a–p** showed that the antifungal activity of chloro-derivatives with $n = 0$ is stronger for the 4-chloro-derivative, followed by 3-chloro- and 2-chloro – which is the opposite of what was observed for antibacterial activity. The addition of one more methylene group ($n = 1$) to the side chain led to changes in the activity of compounds, with the order of activity being 3-Cl > 2-Cl > 4-Cl.

In the series of fluoro derivatives with $n = 0$, the activity follows the order mentioned above for chloro derivatives with $n = 1$.

In the case where $n = 0$, disubstitution of fluorine is preferable (2,6-difluoro- > 2,6-dichloro- > 2-chloro-6-fluoro-) while substitution with two different halogens seems to be more favourable when $n = 1$.

2.3. Elucidating the relationship between antimicrobial activity and molecular properties of synthesized compounds

The predicted values for the physically significant descriptors and pharmaceutically relevant properties are provided in ESI† Table S1. Multivariate data analysis was implemented to elucidate the relation between selected molecular properties of the synthesized compounds and their antimicrobial activities. A PCA model was extracted (Fig. 1) showing three distinct groups of compounds. More specifically, intermediate compounds **3h**, **i**, **j**, **o**, and **p** were located at the second quadrant close to the edge of the model due to their significantly different scaffolds compared to compounds **4a–p**. Interestingly, compounds **4a–p** were divided into two groups: one at the third and one at the first quadrant. The first group (**4-i**) included both the *R* and *S* enantiomers of **4m**, **4o**, and **4p** and only the *S* enantiomers of **4k**, **4l** and **4n**. The second group (**4-ii**) included both the *R* and *S* enantiomers of **4a**, **4b**, **4c**, **4d**, **4e**, **4f**, **4g**, **4h**, **4i**, and **4j** and only the *R* enantiomers of **4k**, **4l** and **4n**. At first glance, there is a spontaneous discrimination between more and less active compounds with respect to their antifungal properties, with the three most active antifungal compounds **4p**, **4m** and **4o** constituting group (**4-i**).

This suggests that **4p**, **4m** and **4o** probably exhibit similarly potent biological activities explained by some common descriptors.

A supervised OPLS-DA was then extracted between groups (**4-i**) and (**4-ii**) (Fig. 1B). The volcano plot highlights the descriptors that contribute to the clustering between the two groups. From these, the most significant parameters for group (**4-i**) are (a) the hydrophobic component of the solvent accessible surface area – SASA (FOSA), (b) the hydrophilic component of the SASA (FISA), (c) the van der Waals surface area of polar nitrogen and oxygen atoms and carbonyl carbon atoms (PSA), (d) the predicted aqueous solubility (QPlogS), (e) the computed dipole moment of the molecule (dipole), (f)

Fig. 1 (A) PCA-X ($A = 6$, $N = 37$, $R^2X(\text{cum}) = 0.99$, $Q^2(\text{cum}) = 0.97$) of synthesized compounds **4a–p** and **3h**, **i**, **j**, **o**, and **p** based only on the predicted physicochemical properties; (B) OPLS-DA-X ($A = 1 + 1$, $N = 32$, $R^2X(\text{cum}) = 0.65$, $R^2Y(\text{cum}) = 0.98$, $Q^2(\text{cum}) = 0.97$) of synthesized compounds **4a–p**.

the square of the dipole moment divided by the molecular volume which is the key term in the Kirkwood–Onsager equation for the free energy of solvation of a dipole with volume V (dip^2/V), (g) the globularity descriptor (glob), (h) the PM3 calculated ionization potential in eV (IP), (i) the predicted maximum transdermal transport rate (J_m) and (j) the predicted IC_{50} value for blockage of HERG K^+ channels (QPlogHERG). As can be observed, these physicochemical parameters are related to the shape, lipophilicity, charge distribution and ionization potential of the molecules. These produced values can be further used as descriptors for QSAR and *in silico* screening techniques.

Regarding the antibacterial activity of the studied compounds, no clear evidence could be extracted from the PCA model.

2.4. Docking-based prediction of the mechanism of antimicrobial activity

2.4.1. Antibacterial activity. Antibacterials may act on several drug targets inhibiting cell wall biosynthesis, protein synthesis, and nucleic acid biosynthesis or disturbing the cell membrane normal function. According to the literature, thiazolyl derivatives have been found to act both on cell wall biosynthesis and DNA replication.^{17,18} Meanwhile, MurA, the first of the six enzymes of the family to be involved in the biosynthesis of cell wall peptidoglycan, is the drug target of the antibiotic fosfomycin; thiazolidinones have been found to inhibit the second enzyme of this process, MurB.^{18,19} On the other hand, several benzothiazole derivatives have been mentioned in the literature as gyrase inhibitors.¹⁷ Based on the structural similarities of our compounds to these inhibitors, we theoretically evaluated the probability of our compounds to act as MurB or gyrase inhibitors, using docking analysis. The crystal structures of *E. coli* MurB 2Q85 and *E. coli* gyrase 4TMA were used for the analysis, and the results are shown in Table 3.

According to prediction results, all compounds could act as *E. coli* MurB or *E. coli* gyrase inhibitors. The estimated binding energy to MurB varied between -9.1 Kcal per mole and -6.1 kcal per mole and to *E. coli* gyrase between -8.2 Kcal per mole and -6.5 Kcal per mole. The two lowest estimated energies to *E. coli* MurB (-9.1 kcal per mole and -8.1 kcal per mole, respectively) were calculated for compounds **4f** and **4h** which exhibited the best antimicrobial activity against *E. coli* (MIC = 10.2). The best estimated binding energy to *E. coli* gyrase (-8.2 kcal per mole) was also calculated for compound **4f**. However, the antimicrobial activity cannot be explained for all the compounds by means of the estimated binding energy to MurB and gyrase.

Although in some cases, such as the third and fourth most active compounds **4a** and **4c** (MIC 11.2 and 16.0, respectively), the poor binding to MurB (est. binding energy -6.9 kcal per mole and -7.5 kcal per mole, respectively) may be balanced by the stronger binding to gyrase (estimated binding energies -7.8 kcal per mole and -8.1 kcal per mole, re-

Table 3 *In vitro* measurement of minimal inhibitory concentration, MIC, against *E. coli* and estimated binding energy (kcal per mole) to *E. coli* MurB and *E. coli* gyrase according to docking analysis

Compound	<i>n</i>	Substituent	Estimated binding energy			clog <i>P</i>
			<i>E. coli</i> MIC	<i>E. coli</i> MurB	<i>E. coli</i> gyrase	
4f	0	3-Cl	10.2	-9.1	-8.2	3.623
4h	0	2,6-F ₂	10.2	-8.1	-7.3	3.766
4a	0	H	11.2	-6.9	-7.8	3.470
4c	0	3-F	16.0	-7.5	-8.1	3.622
4m	1	3-Cl	19.8	-7.2	-6.8	4.183
4l	1	2-Cl	19.8	-6.1	-7.8	4.183
4d	0	4F	21.4	-7.7	-7.0	3.622
4k	1	H	21.6	-6.7	-7.5	3.470
4b	0	2-F	22.5	-7.4	-7.5	3.622
4i	0	2-F,6-Cl	27.0	-7.7	-8.2	4.335
4p	1	2-F,6-Cl	27.0	-7.8	-7.6	4.326
4o	1	2,6-F ₂	27.2	-7.2	-6.9	4.695
4n	1	4-Cl	27.3	-6.6	-6.5	4.182
4e	0	2-Cl	28.2	-7.9	-7.5	4.193
4j	0	2,6-Cl ₂	54.3	-7.6	-7.2	4.906
4g	0	4-Cl	58.9	-7.6	-7.4	4.193
3o	1	2,6-F ₂	11.6	-8.0	-7.9	3.658
3j	0	2,6-Cl ₂	11.6	-7.7	-7.5	3.851
3i	0	2-F,6-Cl	12.0	-7.0	-7.5	3.726
3h	0	2,6-F ₂	25.2	-7.5	-7.7	3.156
3p	1	2-F,6-Cl	36.2	-8.0	-7.4	3.088

spectively); this cannot explain the placement of some less potent antimicrobials in the activity range. For example, compound **4i** with est. binding energies to MurB and gyrase of -7.7 kcal per mole and -8.2 kcal per mole, respectively, was found to be less active (MIC $27.0 \mu\text{mol mL}^{-1} \times 10^{-2}$) than compound **4d** (MIC $21.4 \mu\text{mol mL}^{-1} \times 10^{-2}$) with est. binding energies to MurB and gyrase of -7.7 kcal per mole and -7.0 kcal per mole, respectively. The probable interaction with other targets as well as the permeability of the compound may be responsible for these differences. According to predicted clog *P* values, compound **4i** with a clog *P* 4.335 may exhibit lower permeability than compound **4d** with a clog *P* 3.622. In general, compounds with calculated clog *P* 4.000 exhibited lower antimicrobial activity *in vitro*, although some exceptions were observed (Table 3).

An attempt to explore a probable correlation of the estimated binding energy with both enzymes and antimicrobial activity for the compounds of the thiazolidinone series revealed that such a correlation could poorly follow an exponential curve. The best correlation curve ($R^2 = 0.6829$) for the estimated binding energy against *E. coli* MurB and *E. coli* MIC was obtained for compounds with lower estimated binding energy to MurB compared to gyrase (Fig. S1†). A curve with $R^2 = 0.5934$ was obtained for the correlation of the lowest estimated binding energy against *E. coli* MurB or gyrase (which is lowest) with *E. coli* MIC, for compounds with clog *P* 4.000. Another curve with $R^2 = 0.5571$ and $p = 0.02529$ was obtained for the correlation of the lowest estimated binding energy against *E. coli* MurB or gyrase (which is lowest) with *E. coli* MIC, for all compounds with no clog *P* restrictions.

The results enforced the conception that multiple factors affect activity but suggest a serious involvement mainly of MurB inhibition in the mechanism of action of the most active compounds.

For most of the compounds, the estimated binding energy to MurB was lower than the estimated binding energy to gyrase. Also among the general observations was that carboxamide bridged derivatives, **4a-j**, of the thiazolidinone series, **4a-p**, exhibited better estimated binding energy to MurB than the acetamide bridged compounds, **4k-p**, of this series. In addition, better results were obtained for all substituted carboxamide bridged derivatives compared to the non-substituted one (**4a**), indicating that the presence of halogen atoms at the benzyl ring may favor complex stabilization. Docking analysis revealed that carboxamide bridged derivatives obtain a conformation that enables the aromatic moieties of the molecule to be placed in proximity with the aminoacids Tyr254, Tyr190 and Tyr125 with which they form pi interactions. In addition, hydrogen bonds are formed between halogens of the benzyl ring and Tyr125 or Tyr190 but also with FAD or Pro 252 that further stabilize the complex. The N atom of the thiazolyl ring and the N and O atoms of the thiazolidinone moiety also form hydrogen bonds with Tyr190 and Gly123 in many cases, while the O and N atoms of the carboxamide bridge participate in hydrogen bond formation mainly with Ser229 and Arg159. Polar and other interactions mostly involve Asn233, Tyr158 or Leu218.

According to docking analysis, the greatest number of hydrogen bonds and pi interactions are observed in the predicted complexes of the most active compounds (**4f** and **4h**) against *E. coli*.

More precisely, in the complex stabilization of compound **4f**, the N atoms of the thiazolyl moiety and the carboxamide bridge are predicted to form hydrogen bonds with Gly123 and the O atom of the thiazolidinone is predicted to form hydrogen bonds with Ser229 while the Cl substituent of the benzyl ring interacts with Tyr125. Pi-pi interactions are observed between carbon atoms of the benzothiazole moiety and Tyr190 and Tyr254 and between carbon atoms of the 3Cl-benzyl moiety and Tyr 190, Tyr125 and Tyr254. Ten such pi-pi interactions may be formed (Fig. 2).

Fig. 2 Docking of compounds **4f** and **4c** in the active site of *E. coli* MurB. Tyr254 and carbon 2 of the benzothiazolyl moiety with which it forms pi-pi interactions are in dark yellow.

In an analogue way, five hydrogen bonds can be formed between atoms of the thiazolyl moiety and the carboxamide bridge of compound **4f** and aminoacids Gly123, Ser229, Ala124 and Arg159, while Tyr 254, Tyr190 and Tyr125 may participate in six pi-pi interactions.

Replacement of the Cl substituent of compound **4f** with the F atom in compound **4c** leads to a different orientation with the F atom to interact with Pro254 and the carbon atoms of the 3F-benzyl moiety to form three pi-pi interactions with Tyr254. Two hydrogen bonds are now formed between the N atoms of the thiazolidinone moiety and the carboxamide bridge and hydroxyl group of Tyr190. A much lower number of hydrogen and pi-pi interactions are now formed (Fig. 2).

The additional methylene group at the acetamide bridged derivatives, **4k-p**, of the thiazolidinone series forces them to take a different conformation compared with the carboxamide bridged derivatives. The compounds, either taking a more linear conformation (**4k**) or a more curved conformation (**4o**, **4l**), failed to simultaneously interact with Tyr 254, Tyr190 and Tyr125 which is observed in the case of the best compounds of the carboxamide series. The 2-F,6-Cl derivative, **4p**, with a lower estimated binding energy in the acetamide series, seems to interact with all three tyrosine aminoacids which, probably, play an essential role in complex stabilization (Fig. S2†).

The most active compound in the group of *N'*-(phenylmethylidene)-1,2-benzothiazole-3-carbo/acetohydrazides (**3**) was the 2,6-F₂ derivative (**3o**). Hydrogen bond interactions between N atoms of the acetamide bridge and the OH group of Tyr190 as well as pi-pi interactions between the 2,6-F₂ benzyl ring and Tyr158, and between the benzothiazolyl moiety and Tyr256 seem to play the main role in complex stabilization (Fig. 3).

Docking of the compound (5z)-3-(4-chlorophenyl)-4-hydroxy-5-(1-naphthylmethylene)furan-2(5H)-one, with the ligand bound to MurB in the 2q85 crystal structure and used for docking analysis, showed an estimated binding energy of -8.23 kcal per mole. The main amino acids involved in complex stabilization were Tyr190, Asn233, Pro252 and Lys262, which participated in hydrogen bond formation, and Tyr254, which participated in pi interactions (Fig. S3†).

Docking analysis using *E. coli* gyrase (crystallographic structure, PDB ID: 4TMA) revealed the implication of the

Fig. 3 Docking of compound **3o** in the active site of *E. coli* MurB.

Table 4 Estimated binding energy (kcal per mole) to 14a-lanosterol demethylase (ERG11) of *Candida albicans*

Compound	<i>n</i>	Substituent	C.a. MIC ($\mu\text{M} \times 10^{-2}$)	Estimated binding energy ERG11
4p	1	2-F,6-Cl	4.9	-8.59
4d	0	4-F	8.0	-7.41
4o	1	2,6-F ₂	9.9	-9.15
4m	1	3-Cl	10.0	-8.30
4c	0	3-F	10.7	-7.35
4a	0	H	25.3	-9.30
4j	0	2,6-Cl ₂	39.5	-10.13
4h	0	2,6-F ₂	40.8	-8.88
4i	0	2-F,6-Cl	73.5	-8.09
3j	0	2,6-Cl ₂	23.2	-9.74
3i	0	2-F,6-Cl	24.0	-9.38

aminoacids Val90, Gln94, Ser97, Asp36 and Arg46 in the stabilization of the most potent compounds which is in accordance with the literature (Fig. S4†).²⁰ Docking of novobiocin, a known competitive inhibitor of DNA gyrase, showed an estimated binding energy of -8.4 and the main interactions involved the aminoacids Gln94, Arg91, Phe96 and Ile112.

2.4.2. Anti-fungal activity. Antifungals can be divided into several categories according to the drug targets. Most antifungals target ergosterol synthesis, inhibiting specific enzymes of the biosynthetic pathway. Among these, allylamides and thio-carbamates have been found to inhibit the squalene epoxidase ERG1, several morpholines are known to inhibit ERG2 and ERG24–27 enzymes, polyenes inhibit the last enzyme of the pathway ERG4 while azoles mostly act by inhibiting 14a-lanosterol demethylase encoded by the ERG11 gene.²¹

The *Candida albicans* ERG11 enzyme, which is available in the NCBI database (PDB ID: 5v5z), was used in a first attempt to explore the probable mechanism of antifungal action of our compounds using docking analysis. The results are shown in Table 4.

According to the results, all the analyzed compounds may exhibit inhibitory action against 14a-lanosterol demethylase (ERG11). The estimated binding energy obtained for the analyzed compounds varied between -7.35 kcal per mole and -10.13 kcal per mole. Interestingly, the estimated binding energy of the known antifungal fluconazole according to the docking analysis at the same enzyme structure was -7.52 kcal per mole. Docking of compound 4j and fluconazole is shown in Fig. S4†. However, there is no correlation between experimental antifungal activity of the compounds and predicted 14a-lanosterol demethylase (ERG11) inhibition. Although *in vitro* inhibition assay is needed to elucidate the implication of ERG11 inhibition in the mechanism of antifungal activity, the results indicate that ERG11 may be one but probably not the only target of these compounds.

3. Experimental

3.1. Materials and methods

Unless otherwise noted, reagents were obtained from commercial suppliers and were used without purification.

Anhydrous toluene was obtained by distillation from Na, and anhydrous CH₂Cl₂ was obtained by distillation from calcium hydride. Melting points were measured using Büchi 512 apparatus (Büchi Italia, Milano, Italia) and are uncorrected. Progress of reaction was monitored by TLC with F254 silica-gel precoated sheets (Merck, Darmstadt, Germany). UV light was used for detection. Flash chromatography was performed using Merck silica gel 60 (Si 60, 40–63 μm , 230–400 mesh ASTM). Elemental analyses for C, H, and N were performed using a ThermoQuest Flash 1112 elemental analyser (Thermoquest Italia, Milano, Italy). The percentages found were within $\pm 0.4\%$ of the theoretical values. IR spectra were recorded on a Jasco FT-IR 300E spectrometer (Jasco Europe, Carpi MO, Italia). ¹H-NMR and ¹³C NMR spectra were recorded on a Bruker Avance 400 spectrometer (Bruker Italia, Milano, Italia). The spectra were acquired from samples in DMSO-*d*₆. Chemical shifts are reported as δ (ppm) relative to tetramethylsilane; coupling constants (*J*) are expressed in Hz.

3.2. General procedure for the synthesis of *N'*-(halophenylmethylidene)-1,2-benzothiazole-3-carbohydrazides 3h–j and *N'*-(halophenylmethylidene)-1,2-benzothiazole-3-acetohydrazides 3o–q

A solution of 1,2-benzothiazole-3-carbohydrazide **1** or 1,2-benzothiazole-3-acetohydrazide **2** (6 mmol) and an equimolar amount of the requisite aldehyde in anhydrous ethanol (60 mL) was heated to reflux for 3 hours in the presence of acetic acid (1 mL). The mixture was cooled to room temperature, and the white precipitate that formed was filtered off, washed with water and then recrystallized.

3.2.1. *N'*-(2,6-Difluorophenyl)methylidene]-1,2-benzothiazole-3-carbohydrazide (3h). Mp: 298 °C (DMSO), yield 68%. TLC: CH₂Cl₂/EtOH, 98:2. FTIR (KBr, ν , cm⁻¹): 3153 (NH), 1660 (C=O). ¹H NMR (DMSO-*d*₆, δ , ppm): 12.54 (s, 1H, NH); 8.78–8.74 (m, 2H, CH) 8.35 (d, 1H, *J* = 9, Ar); 7.74–7.49 (m, 3H, Ar); 7.30–7.19 (m, 2H, Ar); ¹³C NMR (DMSO-*d*₆, δ , ppm): 172.68 (C=O); 163.10 (C=N); 152.09 (C); 141.96 (C); 137.65 (CH); 135.26 (C); 132.84 (C); 131.36 (C); 128.39 (CH); 126.68 (CH); 124.52 (CH), 123.48 (CH); 120.94 (CH).

3.2.2. *N'*-(2-Chloro-6-fluorophenyl)methylidene]-1,2-benzothiazole-3-carbohydrazide (3i). Mp: 275 °C (DMSO), yield 94%. TLC: CH₂Cl₂/EtOH, 98:2. FTIR (KBr, ν , cm⁻¹): 3176 (NH), 1662 (C=O). ¹H NMR (DMSO-*d*₆, δ , ppm): 12.65 (s, 1H, NH); 8.87 (s, 1H, CH); 8.74 (d, 1H, *J* = 9, Ar); 8.35 (d, 1H, *J* = 9, Ar); 7.74–7.61 (m, 2H, Ar); 7.54–7.30 (m, 3H, Ar); ¹³C NMR (DMSO-*d*₆, δ , ppm): 170.58 (C=O); 161.82 (C=N); 150.64 (C); 138.44 (C); 136.75 (C); 135.04 (C); 134.09 (CH); 132.34 (C); 124.54 (CH); 123.09 (CH); 120.40 (CH), 112.10 (CH).

3.2.3. *N'*-(2,6-Dichlorophenyl)methylidene]-1,2-benzothiazole-3-carbohydrazide (3j). Mp: 263–265 °C (DMSO), yield 64%. TLC: CH₂Cl₂/EtOH, 98:2. FTIR (KBr, ν , cm⁻¹): 3170 (NH), 1660 (C=O). ¹H NMR (DMSO-*d*₆, δ , ppm): 12.70 (s, 1H, NH); 8.81 (s, 1H, CH); 8.74 (d, 1H, *J* = 9, Ar); 8.35 (d, 1H, *J* = 9,

Ar); 7.74–7.58 (m, 4H, Ar); 7.47 (t, 1H, $J = 9$, Ar); ^{13}C NMR (DMSO- d_6 , δ , ppm): 171.12 (C=O); 167.88 (C=N); 151.12 (C); 137.45 (C); 135.68 (C); 132.28 (C); 130.75 (CH); 127.80 (CH); 125.43 (CH); 124.33 (CH); 123.89 (CH); 120.64 (CH).

3.2.4. N' -[(2,6-Difluorophenyl)methylidene]-1,2-benzothiazol-3-ylacetohydrazide (3o). Mp: 190–192 °C (THF), yield 90%. TLC: $\text{CH}_2\text{Cl}_2/\text{EtOH}$, 95:5. FTIR (KBr, ν , cm^{-1}): 3183 (NH), 1670 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 11.82 (s, 1H, NH); 8.29 (s, 1H, CH); 8.17 (d, 1H, $J = 9$ Ar); 8.10 (d, 1H, $J = 9$ Ar); 7.65–7.28 (m, 5H, Ar); 4.50 (s, 2H, CH_2); ^{13}C NMR (DMSO- d_6 , δ , ppm): 170.75 (C=O); 161.09 (C=N); 152.12 (C); 140.69 (C); 138.66 (CH); 135.26 (C); 133.86 (C); 132.09 (C); 128.39 (CH); 126.77 (CH); 125.34 (CH); 124.65 (CH); 120.87 (CH); 35.66 (CH_2).

3.2.5. N' -[(2-Chloro-6-fluorophenyl)methylidene]-1,2-benzothiazol-3-ylacetohydrazide (3p). Mp: 203–205 °C (EtOH), yield 60%. TLC: $\text{CH}_2\text{Cl}_2/\text{EtOH}$, 95:5. FTIR (KBr, ν , cm^{-1}): 3187, (NH), 1668 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 11.77 (s, 1H, NH); 8.21–8.17 (m, 2H, CH); 8.11 (d, 1H, $J = 9$ Ar); 7.65–7.43 (m, 3H, Ar); 7.21–7.14 (m, 2H, Ar); 4.51 (s, 2H, CH_2); ^{13}C NMR (DMSO- d_6 , δ , ppm): 170.87 (C=O); 164.71 (C=N); 151.90 (C); 138.90 (C); 137.77 (C); 135.28 (C); 134.06 (CH); 132.03 (C); 125.45 (CH); 124.62 (CH); 120.92 (CH); 112.90 (CH); 35.86 (CH_2).

3.2.6. N' -[(2,6-Dichlorophenyl)methylidene]-1,2-benzothiazol-3-ylacetohydrazide (3q). Mp: 230–232 °C (THF), yield 93%. TLC: $\text{CH}_2\text{Cl}_2/\text{EtOH}$, 95:5. FTIR (KBr, ν , cm^{-1}): 3183 (NH), 1668 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 11.86 (s, 1H, NH); 8.29 (s, 1H, CH); 8.17 (d, 1H, $J = 9$ Ar); 8.09 (d, 1H, $J = 9$ Ar); 7.65–7.36 (m, 5H, Ar); 4.28 (s, 2H, CH_2); ^{13}C NMR (DMSO- d_6 , δ , ppm): 171.01 (C=O); 169.91 (C=N); 151.90 (C); 138.90 (C); 135.21 (C); 134.35 (C); 131.44 (CH); 129.80 (CH); 128.26 (CH); 125.33 (CH); 124.38 (CH); 120.88 (CH); 39.37 (CH_2).

3.3. General procedure for the synthesis of N -(4-oxo-2-phenyl-1,3-thiazolidin-3-yl)-1,2-benzothiazole-3-carboxamides 4a–j and N -(4-oxo-2-phenyl-1,3-thiazolidin-3-yl)-1,2-benzothiazole-3-ylacetamides 4k–p

A mixture of the requisite hydrazide 3a–p (1.6 mmol) and sulfanylacetic acid (3.2 mmol) was heated to reflux for 48 hours in dry toluene (30 mL) with stirring. The reaction mixture was then cooled to room temperature and, after concentration and filtration, the solid obtained was washed with an aqueous solution of 10% Na_2CO_3 . The crude product was then purified by flash chromatography and recrystallized from an appropriate solvent or directly recrystallized.

3.3.1. N -(4-Oxo-2-phenyl-1,3-thiazolidin-3-yl)-1,2-benzothiazole-3-carboxamide (4a). Mp: 173–175 °C (EtOH), yield 60%. TLC: $\text{CH}_2\text{Cl}_2/\text{EtOH}$, 98:2. FTIR (KBr, ν , cm^{-1}): 3208 (NH), 1700 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 11.14 (s, 1H, NH); 8.56 (d, 1H, $J = 8$ Ar); 8.30 (d, 1H, $J = 8.2$ Ar); 7.69–7.64 (m, 1H, Ar); 7.61–7.53 (m, 3H, Ar); 7.41–7.31 (m, 3H, Ar); 5.99 (s, 1H, CH); 3.96 (dd, 1H, $J = 1.6$ $J = 16$ CH–H'); 3.83 (d, 1H, $J = 16.1$ CH–H). ^{13}C NMR (DMSO- d_6 , δ , ppm):

169.54 (C=O); 160.01 (C=O); 154.54 (C); 153.30 (C); 138.45 (C); 134.12 (C); 129.47 (CH); 128.98 (CH); 128.87 (CH); 128.32 (CH); 126.81 (CH); 125.61 (CH); 121.26 (CH); 62.36 (CH); 29.90 (CH_2).

3.3.2. N -[2-(2-Fluorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-1,2-benzothiazole-3-carboxamide (4b). Mp: 183 °C (EtOH), yield 67%. TLC: $\text{CH}_2\text{Cl}_2/\text{EtOH}$, 98:2. FTIR (KBr, ν , cm^{-1}): 3201 (NH), 1685 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 11.25 (s, 1H, NH); 8.59 (d, 1H, $J = 8$ Ar); 8.32 (d, 1H, $J = 8.1$ Ar); 7.77–7.57 (m, 3H, Ar); 7.44–7.37 (m, 1H, Ar); 7.28–7.17 (m, 2H, Ar); 6.19 (d, 1H, $J = 1.2$ CH); 3.99 (dd, 1H, $J = 1.5$ $J = 15.9$ CH–H'); 3.84 (d, 1H, $J = 15.9$ CH–H); ($J = 16.1$ CH–H'); 3.82 (d, 1H, $J = 15.9$ CH–H); ^{13}C NMR (DMSO- d_6 , δ , ppm): 169.28 (C=O); 160.13, (C=O); 154.47 (C); 153.33 (C); 134.12 (CH); 131.43 (C); 129.89 (CH); 128.89 (CH); 126.84 (CH); 125.87 (C); 125.60 (CH); 125.21 (C); 121.29 (CH); 116.18 (CH); 115.98 (CH); 55.98 (CH); 29.62 (CH_2).

3.3.3. N -[2-(3-Fluorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-1,2-benzothiazole-3-carboxamide (4c). Mp: 179–182 °C (EtOH), yield 73%. TLC: $\text{CH}_2\text{Cl}_2/\text{EtOH}$, 98:2. FTIR (KBr, ν , cm^{-1}): 3207 (NH), 1710 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 11.21 (s, 1H, NH); 8.60 (d, 1H, $J = 8.1$ Ar); 8.32 (d, 1H, $J = 8.1$ Ar); 7.69 (t, 1H, $J = 7.2$ Ar); 7.60 (t, 1H, $J = 7.5$ Ar); 7.46–7.35 (m, 3H, Ar); 7.21–7.15 (m, 1H, Ar); 6.02 (s, 1H, CH); 4.02 (dd, 1H, $J = 1.5$ $J = 16.1$ CH–H'); 3.82 (d, 1H, $J = 15.9$ CH–H); ^{13}C NMR (DMSO- d_6 , δ , ppm): 169.47 (C=O); 160.09 (C=O); 154.44 (C); 153.33 (C); 141.76 (C); 134.12 (CH); 131.00 (C); 128.89 (CH); 126.84 (CH); 125.60 (CH); 124.38 (CH); 121.30 (CH); 116.40 (CH); 114.88 (CH); 61.53 (CH); 29.73 (CH_2).

3.3.4. N -[2-(4-Fluorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-1,2-benzothiazole-3-carboxamide (4d). Mp: 208 °C (EtOH), yield 62%. TLC: $\text{CH}_2\text{Cl}_2/\text{EtOH}$, 98:2. FTIR (KBr, ν , cm^{-1}): 3224 (NH), 1714 (C=O), 1673 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 11.13 (s, 1H, NH); 8.58 (d, 1H, $J = 8.1$ Ar); 8.32 (d, 1H, $J = 8.1$ Ar); 7.70–7.59 (m, 4H, Ar); 7.20 (t, 2H, $J = 8.9$ Ar); 5.99 (s, 1H, CH); 3.98 (dd, 1H, $J = 16$ CH–H'); 3.83 (d, 1H, $J = 16.1$ CH–H). ^{13}C NMR (DMSO- d_6 , δ , ppm): 169.34 (C=O); 159.99, (C=O); 154.49 (C); 153.31 (C); 134.70 (C); 134.11 (C); 130.72 (C); 128.88 (CH); 126.81 (CH); 125.60 (C); 121.28 (CH); 115.92 (CH); 115.71 (CH); 61.65 (CH); 29.85 (CH_2).

3.3.5. N -[2-(2-Chlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-1,2-benzothiazole-3-carboxamide (4e). Mp: 212–213 °C (EtOH), yield 49%. TLC: $\text{CH}_2\text{Cl}_2/\text{EtOH}$, 98:2. FTIR (KBr, ν , cm^{-1}): 3284 (NH), 1685 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 11.31 (s, 1H, NH); 8.62 (d, 1H, $J = 8$ Ar); 8.32 (d, 1H, $J = 8$ Ar); 7.86 (d, 1H, $J = 7.7$ Ar); 7.70–7.58 (m, 2H, Ar); 7.47–7.35 (m, 3H, Ar); 6.32 (s, 1H, CH); 3.99 (dd, 1H, $J = 1.2$ $J = 16.1$ CH–H'); 3.83 (d, 1H, $J = 16$ CH–H); ^{13}C NMR (DMSO- d_6 , δ , ppm): 169.79 (C=O); 160.22 (C=O); 154.54 (C); 153.30 (C); 136.05 (C); 134.13 (C); 132.52 (C); 130.77 (CH); 130.01 (CH); 128.99 (CH); 128.90 (CH); 128.31 (CH); 126.88 (CH); 125.63 (CH); 121.30 (CH); 58.89 (CH); 29.29 (CH_2).

3.3.6. N -[2-(3-Chlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-1,2-benzothiazole-3-carboxamide (4f). Mp: 145 °C (EtOH), yield 72%. TLC: $\text{CH}_2\text{Cl}_2/\text{EtOH}$, 98:2. FTIR (KBr, ν , cm^{-1}): 3218 (NH), 1689 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 11.22 (s, 1H,

NH); 8.59 (d, 1H, $J = 7.9$ Ar); 8.32 (d, 1H, $J = 8.1$ Ar); 7.70–7.57 (m, 3H, Ar); 7.52–7.47 (m, 1H, Ar); 7.42–7.39 (m, 2H, Ar); 6.00 (s, 1H, CH); 4.01 (dd, 1H, $J = 1.7$ $J = 16.1$ CH–H'); 3.82 (d, 1H, $J = 15.8$ CH–H); ^{13}C NMR (DMSO- d_6 , δ , ppm): 169.42 (C=O); 160.06 (C=O); 154.45 (C); 153.33 (C); 141.36 (C); 134.13 (C); 133.70 (C); 130.82 (CH); 129.40 (CH), 128.90 (CH); 128.03 (CH); 127.04 (CH); 126.84 (CH); 125.59 (CH); 121.30 (CH); 61.51 (CH), 29.75 (CH₂).

3.3.7. *N*-[2-(4-Chlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-1,2-benzothiazole-3-carboxamide (4g). Mp: 173–175 °C (EtOH), yield 70%. TLC: CH₂Cl₂/EtOH, 98:2. FTIR (KBr, ν , cm⁻¹): 3228 (NH), 1716 (C=O), 1675 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 11.15 (s, 1H, NH); 8.58 (d, 1H, $J = 8.1$ Ar); 8.31 (d, 1H, $J = 8.1$ Ar); 7.69–7.57 (m, 4H, Ar); 7.43 (d, 2H, $J = 8.4$ Ar); 5.99 (s, 1H, CH); 3.99 (d, 1H, $J = 16$ CH–H'); 3.83 (d, 1H, $J = 16$ CH–H); ^{13}C NMR (DMSO- d_6 , δ , ppm): 169.34 (C=O); 159.99 (C=O); 154.46 (C); 153.33 (C); 137.68 (C); 134.08 (C); 130.34 (CH); 128.97 (CH); 128.89 (CH), 126.82 (CH); 125.60 (CH); 121.29 61.59 (CH), 29.81 (CH₂).

3.3.8. *N*-[2-(2,6-Difluorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-1,2-benzothiazole-3-carboxamide (4h). Mp: 185 °C (EtOH), yield 60%. TLC: CH₂Cl₂/EtOH, 98:2. FTIR (KBr, ν , cm⁻¹): 3235 (NH), 1720 (C=O), 1681 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 11.33 (s, 1H, NH); 8.53 (d, 1H, $J = 8.1$ Ar); 8.32 (d, 1H, $J = 8.2$ Ar); 7.69 (t, 1H, $J = 7.5$ Ar); 7.59 (t, 1H, $J = 7.4$ Ar); 7.50–7.43 (m, 1H, Ar); 7.12 (t, 2H, $J = 9.3$ Ar); 6.22 (s, 1H, CH); 3.93 (d, 1H, $J = 16.2$ CHH'); 3.84 (d, 1H, $J = 15.9$ CH–H); ^{13}C NMR (DMSO- d_6 , δ , ppm): 168 169.19 (C=O); 160.17 (C=O); 154.47 (C); 153.31 (C); 134.10 (C); 132.07 (CH); 128.91 (CH); 126.85 (CH); 125.48 (CH); 121.30 (CH); 114.99 (C), 112.66 (CH); 112.47 (CH); 51.28 (CH); 29.92 (CH₂).

3.3.9. *N*-[2-(2-Chloro-6-fluorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-1,2-benzothiazole-3-carboxamide (4i). Mp: 176 °C (EtOH), yield 37%. TLC: CH₂Cl₂/EtOH, 98:2. FTIR (KBr, ν , cm⁻¹): 3235 (NH), 1716 (C=O), 1685 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 11.35 (s, 1H, NH); 8.54 (d, 1H, $J = 8.1$ Ar); 8.32 (d, 1H, $J = 8.1$ Ar); 7.68 (td, 1H, $J = 1.2$ $J = 7.8$ Ar); 7.60 (td, 1H, $J = 0.9$ $J = 8.1$ Ar); 7.49–7.42 (m, 1H, Ar); 7.36–7.27 (m, 2H, Ar); 6.48 (s, 1H, CH); 3.95 (d, 1H, $J = 15.3$ CH–H'); 3.86 (dd, 1H, $J = 2.2$ $J = 15.6$ CH–H); ^{13}C NMR (DMSO- d_6 , δ , ppm): 168 168.33 (C=O); 160.32 (C=O); 154.53 (C); 153.31 (C); 134.10 (CH); 132.19 (C); 128.92 (CH); 126.86 (CH); 125.62 (CH); 121.31 (CH); 116.67 (C), 55.21 (CH); 30.00 (CH₂).

3.3.10. *N*-[2-(2,6-Dichlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-1,2-benzothiazole-3-carboxamide (4j). Mp: 221–223 °C (EtOH), yield 31%. TLC: CH₂Cl₂/EtOH, 98:2. FTIR (KBr, ν , cm⁻¹): 3365 (NH), 1722 (C=O), 1691 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 11.33 (s, 1H, NH); 8.55 (d, 1H, $J = 7.9$ Ar); 8.32 (d, 1H, $J = 8.2$ Ar); 7.71–7.55 (m, 3H, Ar); 7.47–7.38 (m, 2H, Ar); 6.82 (d, 1H, $J = 1.9$ CH); 4.00 (dd, 1H, $J = 2.2$ $J = 15.7$ CH–H'); 3.92 (d, 1H, $J = 15.6$ CH–H).

3.3.11. *N*-(4-Oxo-2-phenyl-1,3-thiazolidin-3-yl)-1,2-benzothiazol-3-ylacetamide (4k). Flash chromatography; CH₂Cl₂/EtOH, 98:2. Mp: 176–178 °C (CH₃C₆H₅), yield 16%. TLC: EtOAc/Hex, 4:6. FTIR (KBr, ν , cm⁻¹): 3226 (NH), 1716 (C=O),

1666 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 10.65 (s, 1H, NH); 8.16 (d, 1H, $J = 8.2$ Ar); 7.94 (d, 1H, $J = 8.1$ Ar); 7.59 (t, 1H, $J = 7.2$ Ar); 7.43–7.38 (m, 6H, Ar); 5.76 (s, 1H, CH); 4.01 (s, 2H, CH₂); 3.90 (dd, 1H, $J = 1.6$ $J = 16$ CH–H'); 3.72 (d, 1H, $J = 15.9$ CH–H); ^{13}C NMR (DMSO- d_6 , δ , ppm): 169.42 (C=O); 160.06 (C=O); 154.45 (C); 153.33 (C); 141.36 (C); 134.13 (C); 133.70 (CH); 130.82 (CH); 129.40 (CH), 128.90 (CH); 128.03 (CH); 127.04 (CH); 126.84 (CH); 125.59 (CH); 121.30 (CH); 61.51 (CH), 29.75 (CH₂).

3.3.12. *N*-(2-(2-Chlorophenyl)-4-oxo-1,3-thiazolidin-3-yl)-1,2-benzothiazol-3-ylacetamide (4l). Flash chromatography; EtOAc/Hex, 4:6. Mp: 103–105 °C (CH₃C₆H₅/Hex), yield 45%. TLC: EtOAc/Hex, 6:4. FTIR (KBr, ν , cm⁻¹): 3199 (NH), 1722 (C=O), 1664 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 10.84 (s, 1H, NH); 8.16 (d, 1H, $J = 8.2$ Ar); 7.96 (d, 1H, $J = 8.1$ Ar); 7.62–7.35 (m, 6H, Ar); 6.09 (d, 1H, $J = 1.2$ CH); 4.05 (s, 2H, CH₂); 3.88 (dd, 1H, $J = 1.5$ $J = 15.9$ CH–H'); 3.74 (d, 1H, $J = 15$ CH–H); ^{13}C NMR (DMSO- d_6 , δ , ppm): 169.62 (C=O); 167.45 (C=O); 159.93 (C); 152.09 (C); 136.37 (C); 134.81 (CH); 132.66 (CH); 130.82 (CH); 130.29 (CH); 128.30 (CH); 128.70 (C), 128.39 (CH); 128.28 (CH); 125.24 (CH); 124.32 (CH); 120.89 (CH); 58.62 (CH), 36.94 (CH₂); 29.18 (CH₂).

3.3.13. *N*-(2-(3-Chlorophenyl)-4-oxo-1,3-thiazolidin-3-yl)-1,2-benzothiazol-3-ylacetamide (4m). Flash chromatography; CH₂Cl₂/EtOH, 98:2. Mp: 147–148 °C (CH₃C₆H₅), yield 45%. TLC: EtOAc/Hex, 6:4. FTIR (KBr, ν , cm⁻¹): 3280 (NH), 1716 (C=O), 1660 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 10.62 (s, 1H, NH); 8.16 (d, 1H, $J = 8.2$ Ar); 7.96 (d, 1H, $J = 8.2$ Ar); 7.59 (td, 1H, $J = 1.2$ $J = 8.1$ Ar); 7.51 (s, 1H, Ar); 7.46–7.36 (m, 4H, Ar); 5.78 (d, 1H, $J = 1.5$ CH); 4.02 (s, 2H, CH₂); 3.95 (dd, 1H, $J = 1.7$ $J = 15.9$ CH–H'); 3.72 (d, 1H, $J = 15.9$ CH–H); ^{13}C NMR (DMSO- d_6 , δ , ppm): 168 169.27 (C=O); 167.22 (C=O); 159.96 (C); 156.09 (C); 141.71 (C); 134.79 (C); 133.81 (C); 130.99 (CH); 129.44 (CH); 128.30 (CH); 127.88 (CH), 126.84 (CH); 125.26 (CH); 124.37 (CH); 120.89 (CH); 61.12 (CH), 36.94 (CH₂); 29.51 (CH₂).

3.3.14. *N*-(2-(4-Chlorophenyl)-4-oxo-1,3-thiazolidin-3-yl)-1,2-benzothiazol-3-ylacetamide (4n). Flash chromatography; EtOAc/Hex, 4:6. Mp: 168–170 °C (CH₃C₆H₅), yield 45%. TLC: EtOAc/Hex, 6:4. FTIR (KBr, ν , cm⁻¹): 3222 (NH), 1716 (C=O), 1666 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 10.62 (s, 1H, NH); 8.16 (d, 1H, $J = 8.2$ Ar); 7.93 (d, 1H, $J = 8.1$ Ar); 7.62–7.57 (m, 1H, Ar); 7.46–7.38 (m, 5H, Ar); 5.77 (d, 1H, $J = 1.2$ CH); 4.00 (s, 2H, CH₂); 3.90 (dd, 1H, $J = 1.7$ $J = 16$ CH–H'); 3.72 (d, 1H, $J = 15.8$ CH–H); ^{13}C NMR (DMSO- d_6 , δ , ppm): 168 169.18 (C=O); 167.13 (C=O); 159.94 (C); 152.07 (C); 137.92 (C); 134.77 (CH); 134.06 (CH); 130.11 (CH); 129.10 (CH); 128.68 (CH); 128.29 (CH), 125.24 (CH); 124.37 (CH); 120.88 (CH); 124.32 (CH); 120.88 (CH); 61.20 (CH), 36.94 (CH₂); 29.58 (CH₂).

3.3.15. *N*-(2-(3-Chlorophenyl)-4-oxo-1,3-thiazolidin-3-yl)-1,2-benzothiazol-3-ylacetamide (4m). Flash chromatography; CH₂Cl₂/EtOH, 98:2. Mp: 147–148 °C (CH₃C₆H₅), yield 45%. TLC: EtOAc/Hex, 6:4. FTIR (KBr, ν , cm⁻¹): 3280 (NH), 1716 (C=O), 1660 (C=O). ^1H NMR (DMSO- d_6 , δ , ppm): 10.62 (s,

1H, NH); 8.16 (d, 1H, $J = 8.2$ Ar); 7.96 (d, 1H, $J = 8.2$ Ar); 7.59 (td, 1H, $J = 1.2$ $J = 8.1$ Ar); 7.51 (s, 1H, Ar); 7.46–7.36 (m, 4H, Ar); 5.78 (d, 1H, $J = 1.5$ CH); 4.02 (s, 2H, CH₂); 3.95 (dd, 1H, $J = 1.7$ $J = 15.9$ CH–H'); 3.72 (d, 1H, $J = 15.9$ CH–H); ¹³C NMR (DMSO-d₆, δ , ppm): 169.27 (C=O); 167.22 (C=O); 159.96 (C); 156.09 (C); 141.71 (C); 134.79 (C); 133.81 (C); 130.99 (CH); 129.44 (CH); 128.30 (CH); 127.88 (CH); 126.84 (CH); 125.26 (CH); 124.37 (CH); 120.89 (CH); 61.12 (CH); 36.94 (CH₂); 29.51 (CH₂).

3.3.16. *N*-(2-(2,6-Difluorophenyl)-4-oxo-1,3-thiazolidin-3-yl)-1,2-benzothiazol-3-ylacetamide (4o). Flash chromatography; CH₂Cl₂/EtOH, 98:2. Mp: 70–75 °C (EtOH/H₂O), yield 56%. TLC: EtOAc/Hex, 4:6. FTIR (KBr, ν , cm⁻¹): 3214 (NH), 1720 (C=O), 1675 (C=O). ¹H NMR (DMSO-d₆, δ , ppm): 10.85 (s, 1H, NH); 8.18 (d, 1H, $J = 8.2$ Ar); 7.95 (d, 1H, $J = 8.1$ Ar); 7.62–7.55 (m, 1H, Ar); 7.53–7.47 (m, 1H, Ar); 7.45–7.40 (m, 1H, Ar); 7.15 (t, 2H, $J = 9.2$ Ar); 6.08 (s, 1H, CH); 4.03 (s, 2H, CH₂); 3.82 (dd, 2H, $J = 16.4$ $J = 20$ CH–H); ¹³C NMR (DMSO-d₆, δ , ppm): 168.38 (C=O); 167.51 (C=O); 159.89 (C); 152.08 (C); 134.73 (CH); 132.13 (C); 128.30 (CH); 125.31 (CH); 124.14 (CH); 120.91 (CH); 114.91 (C); 112.80 (C); 51.11 (CH); 36.89 (CH₂); 29.67 (CH₂).

3.3.17. *N*-(2-(2-Chloro-6-fluorophenyl)-4-oxo-1,3-thiazolidin-3-yl)-1,2-benzothiazol-3-ylacetamide (4p). Flash chromatography; CH₂Cl₂/EtOH, 98:2. Mp: 105 °C (CH₃C₆H₅/Hex), yield 20%. TLC: EtOAc/Hex, 4:6. FTIR (KBr, ν , cm⁻¹): 3199 (NH), 1720 (C=O), 1681 (C=O). ¹H NMR (DMSO-d₆, δ , ppm): 10.85 (s, 1H, NH); 8.16 (d, 1H, $J = 8.2$ Ar); 7.95 (d, 1H, $J = 8.1$ Ar); 7.59 (t, 1H, $J = 7.6$ Ar); 7.49–7.30 (m, 4H, Ar); 6.29 (s, 1H, CH); 4.03 (s, 2H, CH₂); 3.84–3.82 (m, 2H, CH–H); ¹³C NMR (DMSO-d₆, δ , ppm): 168.45 (C=O); 167.50 (C=O); 159.85 (C); 152.09 (C); 134.75 (C); 132.17 (C); 132.06 (C); 128.28 (CH); 126.21 (CH); 125.30 (CH); 124.29 (CH); 120.89 (CH); 116.74 (CH); 116.52 (CH); 55.23 (CH); 30.89 (CH₂); 29.74 (CH₂).

3.4. Biological evaluation

3.4.1. Antibacterial activity. The following Gram-negative bacteria were used: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), and *Enterobacter cloacae* (human isolate); the following Gram-positive bacteria were used: *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), *Listeria monocytogenes* (NCTC 7973), and *Staphylococcus 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^{22,23} 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⁻¹. 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.

3.4.2. Microdilution test. The minimal 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⁻¹. Compounds to be investigated were dissolved in 5% DMSO solution containing 0.1% Tween 80 (v/v) (1 mg mL⁻¹) and added in broth (LB) medium (100 μ L) with bacterial inoculum (1.0×10^4 CFU per well) to achieve the desired concentrations. The microplates were incubated using a rotary shaker (160 rpm) for 24 h at 37 °C. The lowest concentrations without visible growth (at the binocular microscope) were defined as the concentrations that completely inhibited bacterial growth (MICs). The MBCs were determined by serial sub-cultivation of 2 μ L into microtitre plates containing 100 μ L 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 using 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 positive controls (1 mg mL⁻¹ in sterile physiological saline). 5% DMSO solution was used as a negative control. All experiments were performed in duplicate and repeated three times.

3.4.3. Antifungal activity. For the antifungal bioassays, eight fungi were used: *Aspergillus niger* (ATCC 6275), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus fumigatus* (human isolate), *Aspergillus versicolor* (ATCC 11730), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112), *Trichoderma viride* (IAM 5061), and *Penicillium versicolor* var. *Cyclopium*. 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.²⁴ In order to investigate the antifungal activity of the compounds, a modified microdilution technique was used.^{22–26}

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 μ L 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. Minimal 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⁻¹) and added in broth malt medium with inoculum. The microplates were incubated in a rotary shaker (160 rpm) for 72 h at 28 °C. The lowest concentrations without visible growth (at the binocular microscope) were defined as the MICs. The fungicidal concentrations (MFCs) were determined by serial subcultivation of 2 μ L of tested compounds dissolved in medium and inoculated for 72 h into microtiter plates containing 100 μ L of broth per well and further incubation 72

h at 28 °C. The lowest concentration with no visible growth was defined as the MFC indicating 99.5% killing of the original inoculum. 5% DMSO solution was used as a negative control, and commercial fungicides, namely, bifonazole (Srbolek, Belgrade, Serbia) and ketoconazole (Zorkapharma, Šabac, Serbia), were used as positive controls (1–3500 µg mL⁻¹). All experiments were performed in duplicate and repeated three times.

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

The best curves for the correlation of the estimated binding energy with anti-bacterial activity were obtained using the online program www.mycurvefit.com.

3.5. Calculation of biologically relevant physicochemical properties

Prediction of the physically significant descriptors and pharmaceutically relevant properties of the novel synthesized compounds **4a–p** and the new intermediates (**3h**, **i**, **j**, **o**, **p**) was performed using the QikProp module of Maestro 9.8 from Schrödinger software. For compounds **4a–p** both the *R* and *S* enantiomers were evaluated.

3.6. Multivariate data analysis

The calculated molecular properties of the novel compounds **4a–p** and **3h**, **i**, **j**, **o** and **p** in relation to their antifungal and antibacterial activities were further subjected to multivariate data analysis. SIMCA-P version 14.0 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 extracts the dominant patterns in the data matrix in terms of a complementary set of scores and loading plots. This approach enables reduction of dimensionality, data exploration finding relationships between objects, estimation of the correlation structure of the variables and investigation on the necessary components (a linear combination of original features) in order to explain the greater part of variance with a minimal loss of information.²⁷ The fraction of the variation of Y that can be predicted by the model was extracted according to the internal cross validation default method of SIMCA-P software.²⁸

Then, classification analysis ensued by further subjecting the data set to orthogonal partial least squares-discriminant analysis (OPLS-DA). OPLS-DA is an extension of the supervised partial least squares (PLS) regression method that manages to increase the quality of the classification model by separating the systematic variation in X into two parts, one that is linearly related to Y (predictive information) and one that is unrelated to Y (orthogonal information). The concept of these methods has been extensively discussed.²⁷

The PCA and OPLS-DA models were UV scaled, extracted at a confidence level of 95% and any observations at <5% were considered to be outliers.

A loading plot was extracted to reveal the variables that have class discriminating power. The volcano plot for the OPLS-DA model was based on feature selection, which included scores larger than 1 for the variable importance in projection (VIP) values and $p(\text{corr}) > 0.4$ to reveal the variables which bear class discriminating power.

The quality of the models was described by the goodness-of-fit R^2 ($0 \leq R^2 \leq 1$) and the predictive ability Q^2 ($0 \leq Q^2 \leq 1$) values. The R^2 explains the variation thus constituting a quantitative measure of how well the data of the training set are 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 which was extracted according to the internal cross validation default method of SIMCA-P software.²⁸

3.7. Docking studies

Docking analysis to the *E. coli* enzyme MurB (PDB ID: 2Q85) and to the *Candida Albicans* 14a-lanosterol demethylase (ERG11) (PDB ID: 2v2z) was carried out using DockingServer.²⁹ Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools.³⁰ AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method.³¹ The initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 100 different runs that were set to terminate after a maximum of 2 500 000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å and quaternion and torsion steps of 5 were applied. Co-enzymes, such as FAD, in MurB were kept in the structure. The pH for ligand preparation was set at 7.0 in all cases.

Docking analysis to *E. coli* gyrase (PDB ID: 4TMA) was performed using the program PyRx based on Autodock Vina.³²

4. Conclusion

The newly synthesized *N*-(2-phenyl-4-oxo-1,3-thiazolidin-3-yl)-1,2-benzothiazole-3-carboxamides and acetamides exhibit remarkable inhibition of the growth of a wide spectrum of Gram positive and Gram negative bacteria and fungi. Almost all our compounds exhibited better than or comparable anti-bacterial activity to reference drugs with the most potent among them being compound **4d** followed by **4h**. The most sensitive bacterial species for these compounds were *S.*

aureus and *E. coli* while *L. monocytogenes* and *S. typhimurium* were the more resistant ones.

As far as fungi are concerned, the tested compounds possess excellent activity against all the fungal species tested, being more than 12 times more active than ketoconazole and 3 times more active than bifonazole. The most sensitive fungi were *T. viride* and *A. ochraceus*, while *A. fumigatus* was the most resistant. The most promising is compound **4p** followed by **4m** and **4o**. A structure–antifungal activity relationship based on predicted physicochemical properties indicated the importance of shape together with the lipophilicity and charge distribution parameters of the studied molecules.

It can be seen that the growth of the tested bacteria (Gram (–) and Gram (+) bacteria) and fungi responded differently to the tested compounds, which indicates that different components may have different modes of action or that the metabolism of some bacteria/fungi is able to better overcome the effect of the compounds or adapt to it. Gram (–) bacteria and fungi are in general more resistant than Gram (+) bacteria.

Docking analysis using *E. coli* MurB and gyrase crystal structures indicated the probable involvement mainly of MurB inhibition and to a lesser extent gyrase inhibition in the mechanism of action of the antibacterial activity of these compounds.

Docking analysis to the antifungal target 14a-lanosterol demethylase (ERG11) revealed that, although *in vitro* assays are needed ERG11 may be one but probably not the only target of these compounds.

Conflicts of interest

The authors declare no competing interests.

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