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2-Heteroarylimino-5-benzylidene-4-thiazolidinones analogues of 2-thiazolylimino-5-benzylidene-4-thiazolidinones with antimicrobial activity: Synthesis and structure–activity relationship

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Abstract—2-Heteroarylimino-5-benzylidene-4-thiazolidinones, unsubstituted or carrying hydroxy, methoxy, nitro and chloro groups on the benzene ring, were synthesised and assayed in vitro for their antimicrobial activity against Gram positive and Gram negative bacteria, yeasts and mould. The antimicrobial activity of the 2-benzo[*d*]thiazolyl- and of the 2-benzo[*d*]isothiazolyl-imino-5-benzylidene-4-thiazolidinones is, on the whole, lower in comparison with the high activity detected for the derivatives of the 2-thiazolylimino-5-benzylidene-4-thiazolidinone class. Nevertheless most of the benzo[*d*]thiazole analogues display good inhibition of the growth of Gram positive bacilli and staphylococci, including methicillin-resistant *Staphylococcus* strains. Among the 2-benzo[*d*]isothiazole analogues a few derivatives show a strong and selective activity against bacilli. Moreover, it is worth noting that the replacement of the thiazole nucleus for the benzo[*d*]thiazole bicyclic system in the parent 2-(benzo[*d*]thiazol-2-ylimino)thiazolidin-4-one leads to significant antifungal properties against both yeasts and moulds, properties not shown by the analogous 2-thiazolyl- and 2-benzo[*d*]isothiazolyl-imino)thiazolidin-4-ones. The structure-activity relationship of 33 analogues possessing the 2-heteroarylimino-4-thiazolidinone structure is analysed through QSAR models.

1. Introduction

A recent survey of novel small-molecule therapeutics revealed that the majority of them result from an analogue-based approach and that their market value represents two-thirds of all drug sales.[1] Because of this, and given our recent finding of a new class of antibacterial agents, the 2-thiazolylimino-5-benzylidene-4-thiazolidinones **1a**–**1j** (Fig. 1),[2] we decided to extend our research to classes of analogues. Starting from the knowledge of the potential of 4-thiazolidinone ring system, which is a core structure in various synthetic pharmaceuticals displaying a broad spectrum of biological activity, including antibacterial and antifungal properties,[3–8] and on the basis of the assumption that a benzylidene moiety at the 5-position of the 4-thiazolidinone is necessary for the antimicrobial activity, we replaced the 2-thiazole ring with analogous groups.

Our analogue-based design encompasses the synthesis of a series of benzo[*d*]thiazole (benzologues, **2a**–**2j**) and of benzo[*d*]isothiazole (positional isomers of the latter, **3a**–**3j**)[9] derivatives (Fig. 1), to be tested for their antimicrobial properties. Furthermore, we have attempted to rationalise the structure–activity relationship of all the 2-heteroarylimino-5-benzylidene-4-thiazolidinones through a QSAR analysis. To increase the chance of selecting structures with the greatest potential for development, prediction of toxicity has been carried out with the DEREK computer program.

Keywords: 4-Thiazolidinone derivatives; Antibacterial activity/antifungal activity; QSAR; DEREK.
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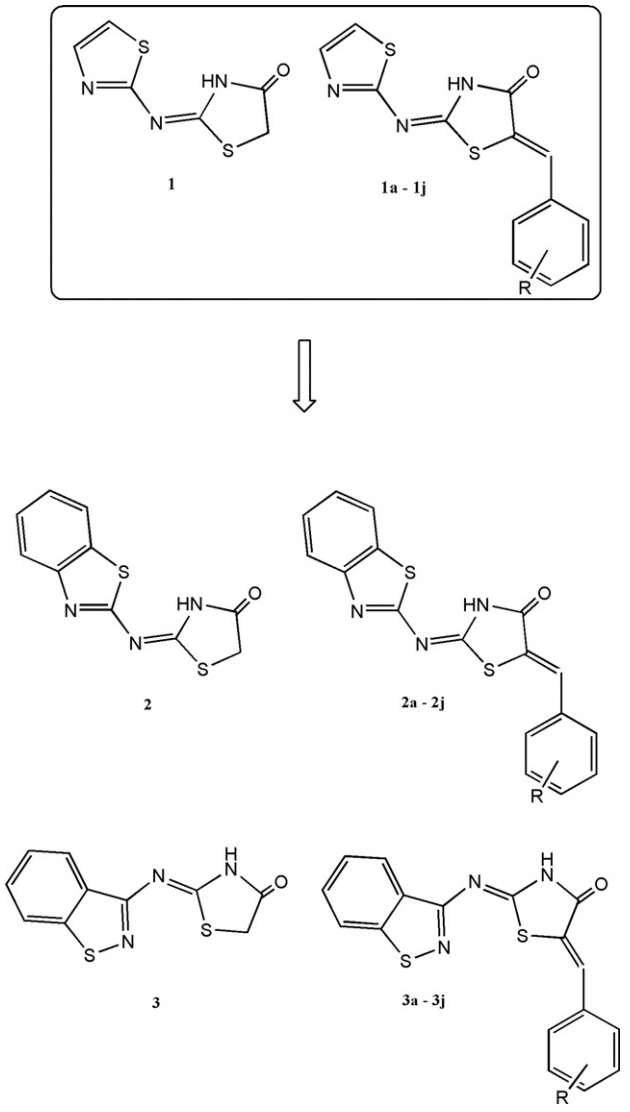

Figure 1. Design of the target compounds **2**, **2a**–**2j** and **3**, **3a**–**3j**. R = H (**a**); 4-OH (**b**); 4-OCH_3 (**c**); 3-OCH_3,4-OH (**d**); 2-NO_2 (**e**); 3-NO_2 (**f**); 4-NO_2 (**g**); 2-Cl (**h**); 3-Cl (**i**); 4-Cl (**j**).

2. Results and discussion

2.1. Chemistry

The compounds described in this paper (Fig. 1) were synthesised by the multi-step reaction protocol reported earlier by us.[2]

The synthetic procedures (Fig. 2) were carried out by reacting the appropriate amine with chloroacetylchloride in *N,N*-DMF at room temperature. After completion of the reaction, which is usually two hours, cyclisation of 2-chloro-*N*-(etheroaryl)acetamide[10–12] in the presence of ammonium thiocyanate, in refluxing ethanol, afforded in excellent yield and purity the 2-(heteroarylimino)thiazolidin-4-ones **1, 2** and **3**.[13,14] The final compounds **1a**–**1j**, **2a**–**2j** and **3a**–**3j** were obtained by refluxing the previous intermediates with commercially available aldehydes and anhydrous sodium acetate in glacial acetic acid. During the course of this study we obtained 2-(benzothiazolylimino)thiazolidin-4-one **2** in good yield through a different procedure from that described by Indian authors who started from benzothiazolylthiourea.[14] The same authors reported in their paper also some of the 2-(benzothiazolylimino)thiazolidin-5-benzylidene-4-ones included in this study, but the physico-chemical data and information on the antibacterial properties of these compounds are not correct, or not understandable, or not comparable to the ones obtained by us, while the experimental protocols are lacking in details. We shall point out in the experimental section the previously cited compounds. The spectral data and the elemental analysis of the new compounds of series **2** and **3** reported in this study correlate with the proposed structures.

The mechanism suggested for the formation of all the compounds under study is presented in Figure 3 for compounds **2** and **2a**–**2j**. The mechanism of the cyclocondensation step, the amino-imino tautomeric equilibrium of the heteroarylthiazolidinones and of their benzylidene derivatives **2a**–**2j** and **3a**–**3j** and the *E*/*Z* potential isomerism of the latter, was investigated through the analysis of ^{1}H NMR and IR spectral data and were confirmed on the basis of the literature data.[2,15]

In the ^{1}H NMR spectra of all the compounds of series **2** and **3**, a NH proton appears at 12.18–13.06 ppm, accounting for a lactam proton but not for an imine proton which is expected around 9.70 ppm. This was considered to be a strong confirmation for the ring closure shown in Figure 3 (step a). Moreover, these low field NH signals for compounds **2**, **2a**–**2j**, **3** and **3a**–**3j**, as well as for **1** and **1a**–**1j**, account for one of the imino tautomeric form shown in Figure 3 (step a). The *Z* configuration of the exocyclic C=C bond, in the 5-benzylidene derivatives **2a**–**2j** and **3a**–**3j**, was attributed on the basis of ^{1}H NMR spectral analysis, since the methine proton resonated, as expected, at higher chemical shift values due to the deshielding effect of the adjacent C=O, than it would do in *E* isomers, because of the lower deshielding effect of 1-S (Fig. 3, step b).

In the solid state, the feature of a γ-lactam heterocycle for the majority of compounds of series **2** and **3** is supported by the IR spectral data showing a NH group as a multiple band near 3100 cm^{-1} and a strong band of the C=O group in the 1725–1687 cm^{-1} region. Compound **3c** is one exception because it shows a single NH absorption band at lower frequencies (3234 cm^{-1}), typical of a secondary amine.

2.2. Antimicrobial activity

The 2-(heteroarylimino)thiazolidin-4-ones **2** and **3** and their 5-benzylidene derivatives **2a**–**2j** and **3a**–**3j** were assayed in vitro for their antimicrobial activity against Gram positive and Gram negative bacteria, yeasts and moulds and the minimal inhibitory concentrations that inhibited the growth of the tested microorganisms (MIC) were detected. The results of antimicrobial testing against a panel of selected Gram positive bacteria and Gram negative *Haemophilus influenzae* are reported in Table 1, in comparison with those of the reference drug ampicillin and of the analogues **1** and **1a**–**1j**.[2]

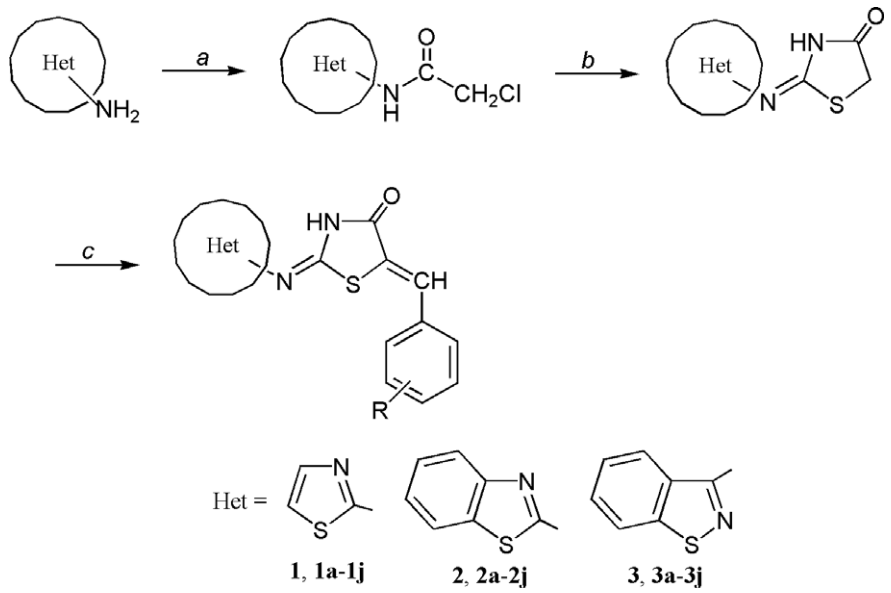

Figure 2. Scheme of synthesis. Reagents and conditions: (a) $ClCOCH_2Cl$, *N*,*N*-DMF, rt; (b) NH_4SCN, EtOH, reflux; (c) RC_6H_4CHO, MeCOOH, MeCOONa, reflux.

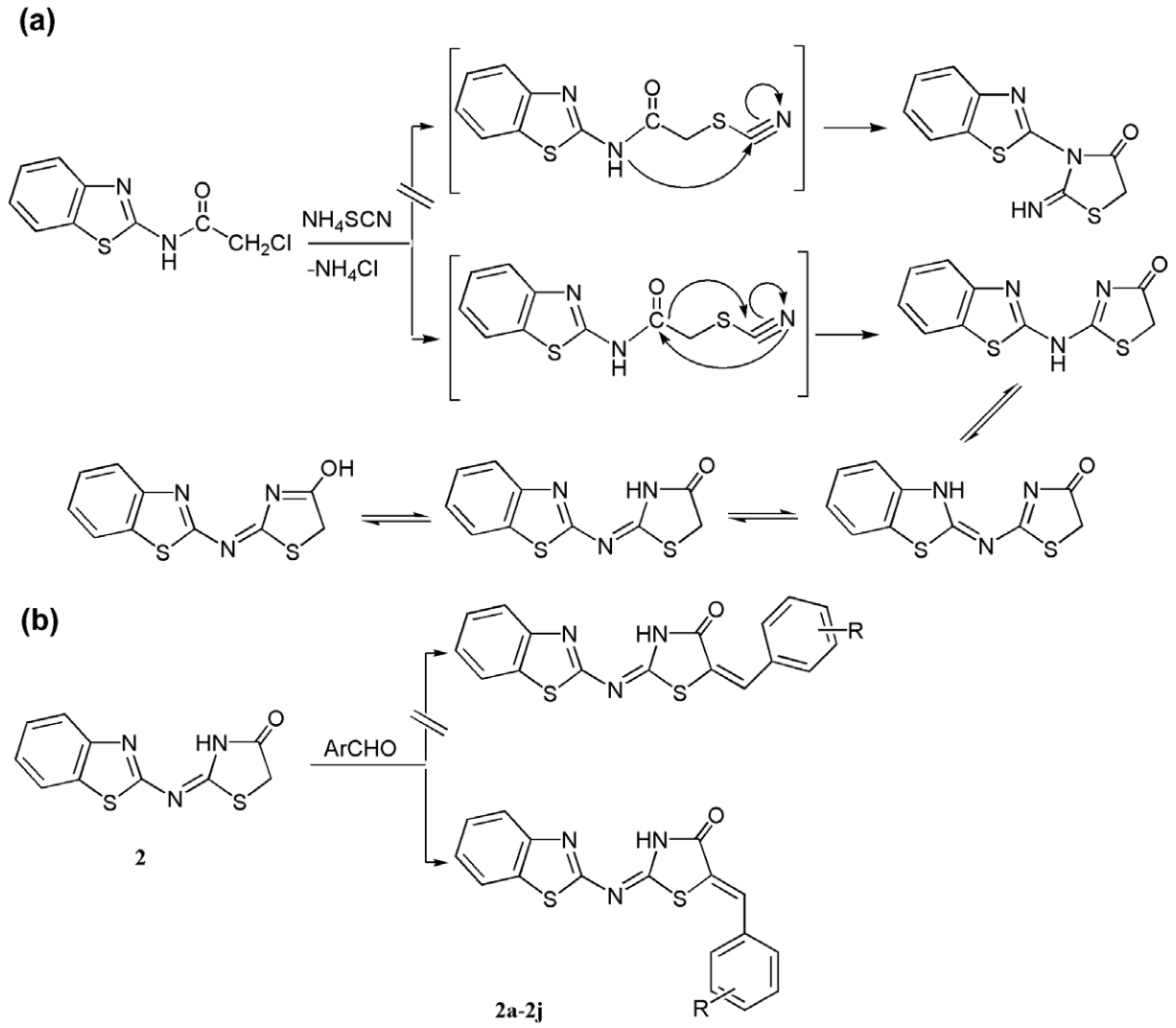

Figure 3. (a) Mechanistic pathway for compound **2** and its tautomers; (b) the *E*/*Z* isomerism of compounds **2a**–**2j**.

Table 1. Antibacterial activity of compounds **1**, **1a**–**1j**, **2**, **2a**–**2j**, **3**, **3a**–**3j** expressed as MIC (μg/mL)

Compound	Bacteria[a]													
	BS	BM	BT	SL	SA	SAR	SE	SER	SH	SAG	SF	SFU	SP	HI
1[2]	50	—[b]	—	—	>100	—	—	—	—	—	—	—	—	1.5
1a[2]	0.7	1.5	3	3	3	25	6	50	6	3	25	25	>100	0.7
1b[2]	3	6	6	12	25	12	25	25	25	12	25	25	25	1.5
1c[2]	1.5	1.5	3	6	12	3	6	12	12	3	>100	>100	12	0.3
1d[2]	3	12	12	12	12	6	12	12	12	6	50	25	25	1.5
1e[2]	6	3	6	12	6	3	12	25	12	12	50	25	25	0.7
1f[2]	3	1.5	3	25	6	1.5	12	12	25	6	50	50	>100	0.7
1g[2]	1.5	0.7	1.5	3	3	1.5	6	6	6	1.5	>100	>100	>100	0.3
1h[2]	0.7	0.3	0.7	3	1.5	0.7	3	3	3	3	12	3	12	0.15
1i[2]	0.3	0.15	1.5	1.5	1.5	0.7	1.5	1.5	3	1.5	50	3	12	0.3
1j[2]	0.3	0.03	0.3	0.15	1.5	0.7	0.7	1.5	3	0.3	3	3	3	0.15
2	12	25	25	50	25	25	100	100	50	>100	>100	>100	>100	0.7
2a	>100	—	—	—	>100	—	—	—	—	—	—	—	—	>100
2b	3	6	3	3	6	3	6	12	12	12	100	50	6	6
2c	>100	—	—	—	>100	—	—	—	—	—	—	—	—	>100
2d	>100	12	6	12	6	6	6	12	>100	>100	>100	>100	>100	>100
2e	>100	—	—	—	>100	—	—	—	—	—	—	—	—	>100
2f	12	12	6	6	6	6	6	6	>100	>100	>100	>100	>100	>100
2g	12	3	3	6	25	6	6	12	>100	>100	>100	>100	>100	12
2h	12	12	12	12	12	12	12	25	>100	>100	>100	>100	25	>100
2i	12	12	6	12	25	25	6	12	>100	>100	>100	>100	>100	>100
2j	12	12	6	12	>100	>100	12	12	100	>100	100	>100	>100	>100
3	50	100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	3
3a	>100	—	—	—	>100	—	—	—	—	—	—	—	—	>100
3b	>100	—	—	—	>100	—	—	—	—	—	—	—	—	>100
3c	>100	—	—	—	>100	—	—	—	—	—	—	—	—	>100
3d	>100	—	—	—	>100	—	—	—	—	—	—	—	—	>100
3e	>100	>100	3	>100	3	50	>100	>100	>100	>100	100	>100	>100	1.5
3f	>100	0.7	1.5	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	3
3g	0.7	0.7	1.5	>100	>100	>100	50	>100	>100	>100	>100	>100	>100	>100
3h	>100	—	—	—	>100	—	—	—	—	—	—	—	—	>100
3i	>100	—	—	—	>100	—	—	—	—	—	—	—	—	>100
3j	3	0.7	6	>100	>100	>100	>100	>100	>100	50	>100	>100	>100	>100
AMP[c]	0.007	0.07	50	0.0015	0.07	25	3	25	0.03	0.03	0.7	100	0.007	0.07

[a] Gram positive bacteria: BS, *Bacillus subtilis* ATCC 6633; BM, *Bacillus megaterium* BGSC 7A2; BT, *Bacillus thuringiensis* var. *kurstaki* BGSC 4D1; SL, *Sarcina lutea* ATCC 9341; SA, *Staphylococcus aureus* ATCC 25923; SAR, penicillin-resistant *Staphylococcus aureus* (clinical isolate); SE, *Staphylococcus epidermidis* ATCC 12228; SER, penicillin-resistant *Staphylococcus epidermidis* (clinical isolate); SH, *Staphylococcus haemolyticus* (clinical isolate); SAG, *Streptococcus agalactiae* (clinical isolate); SF, *Streptococcus faecalis* (clinical isolate); SFU, *Streptococcus faecium* (clinical isolate); SP, *Streptococcus pyogenes* (clinical isolate). Gram negative bacteria: HI, *Haemophilus influenzae* (clinical isolate).

[b] Not tested. Compounds resulted inactive against BS, SA and HI were not tested against the other bacterial strains.

[c] Ampicillin.

Many compounds display good inhibition of the growth of Gram positive bacilli and staphylococci, including methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* strains. Concerning benzothiazole derivatives **2** and **2a**–**2j**, most compounds exhibit MIC values in the 3–25 μg/mL range. It is noteworthy that, in general, they inhibit in a similar manner both penicillin-susceptible and penicillin-resistant *S. aureus* and *S. epidermidis* bacteria; moreover, it should be noted that compound **2g** is found to be fourfold more potent against *S. aureus* resistant strain (MIC 6 μg/mL) than against the susceptible one (MIC 25 μg/mL). Compound **2b** shows the best antibacterial properties, including its activity against all tested streptococci. Against the latter microorganisms, compounds **2h** and **2j** are the only others active, but their effectiveness is restricted to *Streptococcus pyogenes* (MIC 25 μg/mL) and *Streptococcus faecalis* (MIC 100 μg/mL), respectively. A few benzo[*d*]isothiazole derivatives of series **3** are active and they exhibit mostly a selective effect against bacilli. In fact a very significant inhibitory potency is shown at a concentration of 0.7 μg/mL by compound **3g** against *Bacillus subtilis* and by compounds **3f**, **3g** and **3j** against *Bacillus megaterium*, whereas compounds **3f** and **3g** inhibit *Bacillus thuringiensis* at 1.5 μg/mL. A decreased antibacterial activity can be detected for these compounds with respect to benzothiazole derivatives **2** against staphylococci and streptococci.

Concerning *H. influenzae*, only compounds **2**, **2b**, **2g**, **3**, **3e** and **3f** exert a certain effectiveness, with MIC values ranging from 0.7 to 12 μg/mL.

In most cases the inhibitory potency exhibited by the tested substances is lower than that of ampicillin. Noteworthy exceptions arise with *B. thuringiensis* and both penicillin-resistant *S. aureus* and *S. epidermidis* strains,

which appear to be more sensitive to the compounds of the series **2** and **3** and to benzothiazole derivatives **2**, respectively, than to ampicillin.

None of the tested compounds exhibits any activity against Gram negative *Escherichia coli* SPA 27, yeasts (*Candida tropicalis* ATCC 1369 and *Saccharomyces cerevisiae* ATCC 9763), and moulds (*Aspergillus niger* ATCC 6275) up to a concentration of 100 μg/mL, with the exception only of compound **2** that, notably, shows moderate antifungal properties against both yeasts (MIC 50 μg/mL) and moulds (MIC 25 μg/mL). However the inhibition detected is lower than that exhibited by miconazole standard control (data not shown).

All the active compounds exhibit microbiostatic properties having MBC and MFC values higher than the corresponding MICs (data not shown).

As regards the relationships between the structure of the heterocyclic scaffold and the detected antibacterial properties, benzothiazoles are endowed with a wide spectrum of activity against bacilli and staphylococci, including penicillin-resistant ones, that decreases when the benzothiazole nucleus is replaced by its isomer benzisothiazole. Nevertheless, against bacilli, some benzisothiazole derivatives show a better activity compared to the corresponding benzothiazoles.

In contrast to the remarkable activity of 2-thiazolylimino-5-benzylidene-4-thiazolidinone **1a** against all the tested microorganisms, it was observed that in both benzo- and benzisothiazole thiazolidinones the introduction of the benzylidene moiety, at the position 5 of the thiazolidinone ring, leads to compounds **2a** and **3a** with inhibitory activity lower than that of the parent substances **2** and **3**. The effectiveness detected for the benzylidene derivatives of classes **2** and **3** seems to be governed in part by the presence of the substituents on the benzene ring. Clearly, the introduction of the bulky electron-withdrawing nitro and chloro groups enhances the antibacterial properties with respect to **2a** and **3a**. Surprisingly the most potent and wide-spectrum antibacterial agent is **2b**, having in the benzene *para* position a hydroxy group endowed with an electron-donating and hydrophilic character. On the other hand, the introduction of a methoxy substituent does not influence the activity of compounds **2c** and **3c** with respect to the unsubstituted **2a** and **3a**, whereas the antibacterial potencies decrease from **2b** to **2d**.

It seems also interesting to point out that, among the isomeric chloro substituted compounds **2h**–**2j**, as well as among the nitro substituted **3e**–**3g**, the position of the substituent exerts, in general, a certain effect, even if not unilateral, on the activity against all the microorganisms. Conversely, among the nitro substituted **2e**–**2g**, the *meta* (**2f**) and *para* (**2g**) compound possess the highest activity, whereas, for the chloro substituted compounds **3h**-**3j** it is evident that the *para* derivative **3j** has greater efficacy towards bacilli compared with *ortho* **3h** and *meta* **3i**.

Thus, excepting the parent compound **2**, which is more active than its thiazole analogue **1**, the assayed benzothiazole (series **2**) and benzisothiazole (series **3**) bicyclic derivatives exhibit a lower antibacterial activity when compared with the thiazole analogues (series **1**). This shows the modulating effect of the fused benzene ring for the antibacterial activity of the thiazolidinones under study.

2.3. Prediction of toxicity and QSAR studies

Most of the compounds were predicted by DEREK to have no toxicity (Table 2). For 10 compounds (**1**, **1e**–**1g**, **2e**–**2g**, **3e**–**3g**), carcinogenicity was predicted to be plausible. For most of the compounds the predicted carcinogenicity was related to the presence of an aromatic nitro group. Skin sensitisation was predicted to be plausible for compounds **1d**, **2d** and **3d**, because of the presence of a catechol precursor. These predicted plausible toxicities should not preclude the further investigation of these compounds. However, if any of them is selected for development, the appropriate toxicity tests should be carried out.

Good QSARs were obtained for activities against some bacteria, but not for others. In part this was due to only a small number of compounds yielding quantitative MIC values against certain bacteria. This limits the number of descriptors that can be incorporated into a QSAR, according to Topliss and Costello,[16] which requires the

Table 2. DEREK predictions of compounds **1**, **1a**–**1j**, **2**, **2a**–**2j**, **3**, **3a**–**3j**

Compound	Carcinogenicity	Skin sensitisation
1	Plausible	None
1a	None	None
1b	None	None
1c	None	None
1d	None	Plausible (catechol or precursor)
1e	Plausible	None
1f	Plausible	None
1g	Plausible	None
1h	None	None
1i	None	None
1j	None	None
2	None	None
2a	None	None
2b	None	None
2c	None	None
2d	None	Plausible (catechol or precursor)
2e	Plausible	None
2f	Plausible	None
2g	Plausible	None
2h	None	None
2i	None	None
2j	None	None
3	None	None
3a	None	None
3b	None	None
3c	None	None
3d	None	Plausible (catechol or precursor)
3e	Plausible	None
3f	Plausible	None
3g	Plausible	None
3h	None	None
3i	None	None
3j	None	None

ratio of compounds to descriptors to be at least 5:1, in order to minimise the risk of chance correlations.

In order to be of value, a QSAR must have good statistics, in terms of both its correlation coefficient R and its cross-validated R value, Q. Acceptable values are $R^2 \geqslant 0.7$ and $Q^2 \geqslant 0.5$.[17] The QSARs reported below are those that satisfy those criteria. It is disappointing that an acceptable QSAR could not be obtained for *Bacillus thuringiensis* var. *kurstaki* (BT), since our compounds were found to be potent against this bacillus.

The best QSAR found for the antimicrobial activity, where n, number of compounds used to develop the QSAR; R, multiple correlation coefficient; Q, cross validated multiple correlation coefficient (leave-one-out procedure); s, standard error of estimate and F, Fisher statistic are

Bacillus subtilis (BS)

$$\log(1/C) = -29.1Q^{-}_{\max} - 20.7Q^{+}_{\max} - 3.43\mathrm{SHDW5} + 1.24$$
$$n = 21 \quad R^2 = 0.822 \quad Q^2 = 0.744 \quad s = 0.309 \quad F = 26.2 \tag{1}$$

where $Q^{-}_{\max}$ and $Q^{+}_{\max}$ = maximum negative and positive charges on a molecule, and SHDW5 = normalised shadow area in XZ plane.

The sign of the SHDW5 term indicates that smaller molecules are more active. The $Q^{-}_{\max}$ and $Q^{+}_{\max}$ terms indicate that a high maximum negative charge increases activity, whereas a high maximum positive charge decreases activity.

Bacillus megaterium (BM)

$$\log(1/C) = -1.31\mathrm{RNCS_{AM1}} + 25.7\mathrm{Sum(Ca)}/\alpha + 0.239\mathrm{DPM}_Y - 2.22$$
$$n = 21 \quad R^2 = 0.781 \quad Q^2 = 0.672 \quad s = 0.383 \quad F = 20.3 \tag{2}$$

where $\mathrm{RNCS_{AM1}}$ = relative negatively charged surface area calculated by AM1; Sum(Ca)/α = (sum of hydrogen bond acceptor abilities)/molar polarisability and DPM_Y = dipole moment Y vector.

The negative coefficient of $\mathrm{RNCS_{AM1}}$ indicates that negatively charged surface area lowers activity. On the other hand, hydrogen bond acceptor ability, represented by Sum(Ca)/α, improves activity. Increased dipole moment also improves activity.

Sarcina lutea (SL)

$$\log(1/C) = -15.8\mathrm{SHDW4} + 0.0460\Sigma\mathrm{ESI} + 0.0102\mathrm{Oct_{XYZ}} + 6.71$$
$$n = 18 \quad R^2 = 0.746 \quad Q^2 = 0.575 \quad s = 0.308 \quad F = 13.7 \tag{3}$$

where SHDW4 = shadow area 1 in XY plane, Σ ESI = sum of electrotopological state indices, and Oct_{XYZ} = VAMP octupole in XYZ hyperplane.

The negative sign of SHDW4 indicates that smaller molecules should be more active, whilst Oct_{XYZ} indicates the importance of polarity in governing activity. The Σ ESI term is difficult to interpret, as E-state values incorporate both steric and electronic components; since the range of values of Σ ESI is very small (26–64), it could be that this term is of little significance.

Staphylococcus aureus (SA)

$$\log(1/C) = -0.00156\mathrm{IM2S} + 0.333\mathrm{LP}_Z + 0.00246\mathrm{Oct}_{XYY} + 2.57$$
$$n = 18 \quad R^2 = 0.700 \quad Q^2 = 0.566 \quad s = 0.262 \quad F = 10.9 \tag{4}$$

where IM2S = moment of inertia 2 (size); LP_Z = lipole Z vector and Oct_{XYY} = VAMP octupole in XYY hyperplane.

The negative coefficient of IM2S indicates that smaller molecules are more active. The LP_Z term suggests that lipophilicity improves activity, and, as with HI above, lower values of Oct_{XYY} will enhance activity.

Staphylococcus aureus penicillin-resistant (SAR)

$$\log(1/C) = -0.0527\mathrm{MW} - 1.14\mathrm{IM2L} + 0.228\mathrm{MR} + 3.31$$
$$n = 18 \quad R^2 = 0.752 \quad Q^2 = 0.629 \quad s = 0.318 \quad F = 14.1 \tag{5}$$

where MW = molecular weight; IM2L = moment of inertia 2 (length) and MR = molar refractivity.

MW and IM2L both indicate that smaller molecules have greater activity. MR can also be considered to represent molecular size, but also reflects polarisability. The positive coefficient of this term suggests that a higher proportion of non-polar surface area could enhance activity.

Staphylococcus epidermidis (SE)

$$\log(1/C) = -1.83\mathrm{RNCS_{AM1}} + 1.08E_{\mathrm{HOMO}} + 64.2^{5}\chi_{\mathrm{ch}} - 0.48$$
$$n = 19 \quad R^2 = 0.759 \quad Q^2 = 0.670 \quad s = 0.265 \quad F = 15.7 \tag{6}$$

where E_{HOMO} = energy of highest occupied molecular orbital and $^{5}\chi_{\mathrm{ch}}$ = 5th order chain molecular connectivity.

The negative coefficient of $\mathrm{RNCS_{AM1}}$ indicates that negatively charged surface area lowers activity, whilst E_{HOMO}, *with negative values, indicates that high values also lower activity. This is consistent with the effect of* $RNCS_{\mathrm{AM1}}$, *since* E_{HOMO} is a measure of electron-donating ability. The $^{5}\chi_{\mathrm{ch}}$ term can be considered in effect an indicator variable for the presence of five-membered rings, which clearly enhance activity.

Staphylococcus epidermidis penicillin-resistant (SER)

$$\log(1/C) = -0.876\text{EDIF1} - 60.8\text{HALO4} + 8.34N_{\text{Hal}} + 12.4$$
$$n = 18 \quad R^2 = 0.853 \quad Q^2 = 0.775 \quad s = 0.205 \quad F = 27.1 \tag{7}$$

where EDIF1 = difference between maximum and minimum atomic *E*-state values; HALO4 = (halogen partial atomic surface area)/(total molecular surface area); and N_{Hal} = number of halogen atoms.

E-State values have both steric and electronic components. The negative sign of EDIF1 suggests that smaller molecules should contribute less to reduced activity. HALO4 and N_{Hal} both relate to halogen atoms, but are of opposite sign. Clearly the more halogen atoms the higher the activity. The negative sign of HALO4 suggests that smaller halogen atoms, and/or halogen atoms adjacent to one another, are better for activity.

Staphylococcus haemolyticus (SH)

$$\log(1/C) = -0.0906\text{SRMN} + 0.0212\text{Oct}_{XXZ} + 0.744$$
$$n = 13 \quad R^2 = 0.753 \quad Q^2 = 0.604 \quad s = 0.259 \quad F = 15.3 \tag{8}$$

where SRMN = minimum free radical superdelocalisability; and Oct_{XXZ} = VAMP octupole in *XXZ* hyperplane.

SRMN is a measure of the concentration of orbitals, occupied and unoccupied, at each atom, and can be considered as the electron richness of each atom. Its values are negative, and so the QSPR indicates that electron-rich atoms will enhance activity. The presence of Oct_{XXZ}, with positive values, suggests that increased polarity will aid activity.

Streptococcus agalactiae (SAG)

$$\log(1/C) = -18.9\text{SHDW4} - 48.4\text{RNCG}_{\text{AM1}} - 0.00794\text{IM1S} + 19.3$$
$$n = 12 \quad R^2 = 0.944 \quad Q^2 = 0.864 \quad s = 0.155 \quad F = 44.9 \tag{9}$$

where RNCG_{AM1} = relative negative charge calculated by AM1; and IM1S = whole molecule moment of inertia 1 (size).

SHDW4 and IM1S are both related to molecular size, and both have negative coefficients, indicating that smaller molecules have greater activity. Charge also plays a part, as indicated by the RNCG_{AM1} term; since the RNCG_{AM1} values themselves are positive, the negative sign of the coefficient means that smaller relative negative charge results in increased activity.

Haemophilus influenzae (HI)

$$\log(1/C) = 0.00170\text{Oct}_{YYY} + 41.6\text{FPSA3} - 0.0410\text{Pol}_{\text{XZ}} - 1.99$$
$$n = 17 \quad R^2 = 0.835 \quad Q^2 = 0.629 \quad s = 0.238 \quad F = 21.9 \tag{10}$$

where FPSA3 = fractional positively charged partial surface area; and Pol_{XZ} = VAMP polarisation in *XZ* plane.

All of these descriptors relate to polarity; Oct_{YYY} values are negative, whilst Pol_{XZ} values are positive, so lower values of both will enhance activity. On the other hand, the sign on FPSA3 indicates that increased positively charged surface area will also enhance activity. The two are not mutually exclusive.

The descriptors selected by the software's genetic algorithm (GA) are, with only one exception (the lipophilicity descriptor LP_Z in Eq. 4), those representing electronic and steric effects, and it can be concluded that antimicrobial activity in the compounds studied is controlled largely by these effects. Because of the good statistics of Eqs. 1–10, it should be possible to use each of the QSARs to predict more active compounds against each species.

It can be seen that Eqs. 1–10 involve a wide variety of descriptors. The GA algorithm that we used yielded the best ten QSARs for activity against each species, which allowed us to see whether there were any good QSARs that were common to two or more microbial species. This was found not to be so. We therefore investigated whether the descriptors selected for the best QSAR for one species could be used to develop a good QSAR for other species. This was found not to be the case, from which we conclude that the mechanisms of action are different for each species, although it should be pointed out that several of the QSARs indicate that smaller molecules should be more active, and that polarity is important for activity.

3. Conclusion

We report the antimicrobial activity of novel 2-benzo[*d*]thiazolylimino-5-benzylidene-4-thiazolidinones and their 2-benzo[*d*]isothiazolyl isomers and the comparison of their properties with those of the previous potent analogous thiazolylimino-5-benzylidene-4-thiazolidinones. Most of the benzothiazoles show significant antibacterial efficacy against bacilli and staphylococci, including penicillin-resistant strains, whereas some of the tested benzisothiazoles are selective against bacilli. Overall, the activity depends on the substituents at the 5-benzylidene moiety. On the other hand, both the benzothiazoles and benzisothiazoles exhibit decreased antibacterial properties when compared to the corresponding thiazoles, suggesting that these bicyclic systems play a negative role on the antimicrobial effectiveness of this class of compounds. The QSAR study indicates that the

mechanisms of action are different for each species, although several of the QSARs suggest that smaller molecules should be more active, and that polarity is important for activity.

4. Experimental

4.1. Chemistry

Melting points (mp °C) were determined with a Buchi 512 apparatus or with a Boetius apparatus and are uncorrected. Elemental analysis was performed on a ThermoQuest (Italia) FlashEA 1112 Elemental Analyser, for C, H, N and S. The values found for C, H, N and S were within ±0.4% of the theoretical ones. IR spectra, such as KBr pellets, were recorded on a JASCO FT-IR 300E spectrophotometer (Jasco Ltd, Tokyo, Japan); wave numbers in the IR spectra are given in cm^{-1}. ^1H NMR spectra of the newly synthesised compounds, in DMSO-d_6 solutions, were recorded on a Bruker AC 300 instrument at 298 K. Chemical shifts are reported as δ (ppm) relative to TMS as internal standard; coupling constants J are expressed in Hz. The progress of the reactions was monitored by thin layer chromatography with F_{254} silica-gel precoated sheets (Merck, Darmstadt, Germany). UV light was used for detection.

Solvents, unless otherwise specified, were of analytical reagent grade or of the highest quality commercially available. Synthetic starting material, reagents and solvents were purchased from Aldrich Chemical Co. and from Fluka.

4.1.1. General procedure for synthesis of 2-(heteroarylimino)thiazolidin-4-ones 2 and 3. A solution of 2-chloro-*N*-(etheroaryl)acetamide (5 mmol) and ammonium thiocyanate (10 mmol) in 20 mL of 96% ethanol was refluxed for 1–3 h and allowed to stand overnight. The precipitate was filtered, washed with water and then recrystallised.

4.1.1.1. 2-(Benzo[*d*]thiazol-2-ylimino)thiazolidin-4-one 2.[14] Reaction time: 1 h. Yield: 65%; mp 189–190 °C (dioxane). TLC: eluent = benzene/ethanol 8/2. IR (KBr): ν = 3158 (N–H), 1725 (C=O), 1568 (N=C) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 12.27 (s, 1H, NH); 7.97 (d, 1H, *J*=8.4 H-7); 7.80 (d, 1H, J = 8.1 H-4); 7.45 (t, 1H, J = 8.4 H-5); 7.33 (t, 1H, J = 8.2 H-6); 4.05 (s, 2H, CH_2). Anal. calcd for $C_{10}H_7N_3OS_2$ (249.31): C, 48.18; H, 2.83; N, 16.85; S, 25.72. Found C, 48.38; H, 2.91; N, 16.64; S, 25.87%.

4.1.1.2. 2-(Benzo[*d*]isothiazol-3-ylimino)thiazolidin-4-one 3. Reaction time: 3 h. Yield: 76%; mp 205–206 °C (dioxane). TLC: eluent = CH_2Cl_2/EtOH 95/5. IR (KBr): ν = 3110 (N–H), 1702 (C=O), 1575 (N=C) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 12.18 (s, 1H, NH); 8.13 (d, 1H, J = 8.4, H-4); 8.02 (d, 1H, J = 7.8, H-7); 7.62 (t, 1H, J = 7.5, H-5); 7.51 (t, 1H, J = 7.5, H-6); 4.03 (s, 2H, CH_2). Anal. calcd for $C_{10}H_7N_3OS_2$ (249.31): C, 48.18; H, 2.83; N, 16.85; S, 25.72. Found C, 48.17; H, 2.87; N, 16.89; S, 25.32%.

4.1.2. General procedure for synthesis of 2-heteroarylimino-5-benzylidene-4-thiazolidinones 2a–2j, 3a–3j. A well-stirred solution of 2-(heteroarylimino)thiazolidin-4-one (4 mmol) in 35 mL of acetic acid was buffered with sodium acetate (8 mmol) and added with the appropriate arylaldehyde (6 mmol). The solution was refluxed over different periods till the completion of the reaction, monitoring by TLC. The reaction mixture was then cooled at room temperature and the solid precipitated was filtered, abundantly washed with water and then recrystallised.

4.1.2.1. 2-(Benzo[*d*]thiazol-2-ylimino)-5-benzylidene-thiazolidin-4-one 2a.[14] Reaction time: 5 h. Yield: 52%; mp 281–284 °C (dioxane). TLC: eluent = toluene/dioxane/acetic acid 90/10/5. IR (KBr): ν = 3120 (N–H), 1699 (C=O), 1574 (N=C) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 12.91 (s, 1H, NH); 7.99 (d, 1H, J = 7.8 H-4); 7.92 (d, 1H, J = 7.8 H-7); 7.79 (s, 1H, CH); 7.70 (d, 2H, J = 7.2 H-2′, H-6′); 7.61–7.48 (m, 4H, H-5, H-6, H-3′, H-5′); 7.38 (t, 1H, J = 7.5 H-4′). Anal. calcd. for $C_{17}H_{11}N_3OS_2$ (337.42): C, 60.51; H, 3.29; N, 12.45; S, 19.01. Found C, 60.43; H, 3.44; N, 12.15; S, 19.11%.

4.1.2.2. 5-(4-Hydroxybenzylidene)-2-(benzo[*d*]thiazol-2-ylimino)thiazolidin-4-one 2b.[14] Reaction time: 6 h. Yield: 45%; mp 293–294 °C (dioxane). TLC: eluent = toluene/dioxane/acetic acid 90/10/5. IR (KBr): ν = 3400 (O–H), 3129 (N–H), 1696 (C=O), 1564 (N=C) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 12.79 (s, 1H, NH); 10.38 (s, 1H, OH); 8.00–7.93 (m, 2H, H-4, H-7); 7.70 (s, 1H, CH); 7.58 (d, 2H, J = 8.1 H-2′, H-6′); 7.49 (t, 1H, J = 7.6 H-5); 7.36 (t, 1H, J = 7.6 H-6); 6.98 (d, 2H, J = 8.3 H-3′, H-5′). Anal. calcd for $C_{17}H_{11}N_3O_2S_2$ (353.42): C, 57.77; H, 3.14; N, 11.89; S, 18.15. Found C, 57.40; H, 3.32; N, 12.01; S, 18.10%.

4.1.2.3. 5-(4-Methoxybenzylidene)-2-(benzo[d]thiazol-2-ylimino)thiazolidin-4-one 2c.[14] Reaction time: 4 h. Yield: 60%; mp 223–225 °C (dioxane). TLC: eluent = toluene/dioxane/acetic acid 90/10/5. IR (KBr): ν = 3143 (N–H), 1699 (C=O), 1585 (N=C) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 12.81 (s, 1H, NH); 7.98 (d, 1H, J = 7.8 H-4); 7.91 (d, 1H, J = 7.8 H-7); 7.74 (s, 1H, CH); 7.65 (d, 2H, J = 8.7 H-2′, H-6′); 7.49 (t, 1H, J = 7.5 H-5); 7.36 (t, 1H, J = 7.5 H-6); 7.13 (d, 2H, J = 8.7 H-3′, H-5′); 3.84 (s, 3H, CH_3O). Anal. calcd for $C_{18}H_{13}N_3O_2S_2$ (367.04): C, 58.84; H, 3.57; N, 11.44; S, 17.45. Found C, 58.77; H, 3.69; N, 11.11; S, 17.55%.

4.1.2.4. 5-(4-Hydroxy-3-methoxybenzylidene)-2-(benzo[*d*]thiazol-2-ylimino)thiazolidin-4-one 2d. Reaction time: 4 h. Yield: 83%; mp 178–180 °C (dioxane). TLC: eluent = toluene/dioxane/acetic acid 90/10/5. IR (KBr): ν = 3440 (O–H), 3193 (N–H), 1698 (C=O), 1581 (N=C) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 12.79 (s, 1H, NH); 10.01 (s, 1H, OH); 8.01 (d, 1H, J = 8.1 H-7); 7.89 (d, 1H, J = 7.8 H-4); 7.71 (s, 1H, CH); 7.49 (t, 1H, J = 7.9 H-5); 7.39–7.30 (m, 2H, H-2′, H-6′); 7.21 (d, 1H, J = 8.1, H-6′); 7.00 (d, 1H J = 8.4 H-5′); 3.88 (s, 3H, CH_3O). Anal. calcd for $C_{18}H_{13}N_3O_3S_2$ (383.45): C, 56.38; H, 3.42; N, 10.96; S, 16.73. Found C, 56.52; H, 3.82; N, 10.59; S, 16.96%.

4.1.2.5. 5-(2-Nitrobenzylidene)-2-(benzo[*d*]thiazol-2-ylimino)thiazolidin-4-one 2e. Reaction time: 4 h. Yield: 72%; mp 254–255 °C (dioxane). TLC: eluent = toluene/dioxane/acetic acid 90/10/5. IR (KBr): ν = 3156 (N–H), 1720 (C=O), 1583 (N=C) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 13.03 (s, 1H, NH); 8.24 (d, 1H, J = 8.1 H-3′); 8.01–7.74 (m, 6H, H-4, H-7, H-4′, H-5′, H-6′, CH); 7.46 (t, 1H, J=7.5 H-5); 7.36 (t, 1H, J = 7.5 H-6). Anal. calcd for $C_{17}H_{10}N_4O_3S_2$ (382.42): C, 53.39; H, 2.64; N, 14.65; S, 16.77. Found C, 53.52; H, 3.03; N, 14.49; S, 16.37%.

4.1.2.6. 5-(3-Nitrobenzylidene)-2-(benzo[*d*]thiazol-2-ylimino)thiazolidin-4-one 2f. Reaction time: 4 h. Yield: 81%; mp 255–256 °C (dioxane). TLC: eluent = toluene/dioxane/acetic acid 90/10/5. IR (KBr): ν = 3156 (N–H), 1690 (C=O), 1594 (N=C) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 13.04 (s, 1H, NH); 8.54 (s, 1H, H-2′); 8.29 (d, 1H, J = 8.1 H-4′); 8.10 (d, 1H, J = 8.1 H-6′); 8.01 (d, 1H, J=7.8 H-7); 7.90–7.83 (m, 3H, H-4, H-5, CH); 7.51 (t, 1H, J = 7.6 H-5); 7.38 (t, 1H, J = 7.6 H-6). Anal. calcd for $C_{17}H_{10}N_4O_3S_2$ (382.42): C, 53.39; H, 2.64; N, 14.65; S, 16.77. Found C, 53.29; H, 2.78; N, 14.69; S, 16.87 %.

4.1.2.7. 5-(4-Nitrobenzylidene)-2-(benzo[*d*]thiazol-2-ylimino)thiazolidin-4-one 2g.[14] Reaction time: 4 h. Yield: 84%; mp 288–290 °C (dioxane). TLC: eluent = toluene/dioxane/acetic acid 90/10/5. IR (KBr): ν = 3140 (N–H), 1720 (C=O), 1592 (N=C) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 13.01 (s, 1H, NH); 8.38 (d, 2H, J = 8.7 H-3′, H-5′); 8.01 (d, 1H, J = 7.8 H-7); 7.95–7.93 (m, 3H, H-4, H-2′, H-6′); 7.87 (s, 1H, CH); 7.52 (t, 1H, J = 7.3 H-5); 7.39 (t, 1H, J = 7.7 H-6). Anal. calcd for $C_{17}H_{10}N_4O_3S_2$ (382.42): C, 53.39; H, 2.64; N, 14.65; S, 16.77. Found C, 53.13; H, 2.76; N, 14.31; S, 16.72 %.

4.1.2.8. 5-(2-Chlorobenzylidene)-2-(benzo[*d*]thiazol-2-ylimino)thiazolidin-4-one 2h.[14] Reaction time: 6 h. Yield: 89%; mp 203–205 °C (dioxane). TLC: eluent = toluene/dioxane/acetic acid 90/10/5. IR(KBr): ν = 3152 (N–H), 1698 (C=O), 1587 (N=C) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 13.06 (s, 1H, NH); 8.02 (d, 1H J = 7.5 H-4); 7.91–7.88 (d, 1H, J = 7.2 H-7); 7.76 (d, 1H, J = 7.2 H-6′); 7.68–7.46 (m, 5H, H-5, H-3′, H-4′, H-5′, CH); 7.37 (t, 1H, J = 7.3 H-6). Anal. calcd for $C_{17}H_{10}ClN_3OS_2$ (371.87): C, 54.91; H, 2.71; N, 11.30; S, 17.25. Found C, 54.97; H, 3.04; N, 10.91; S, 17.55%.

4.1.2.9. 5-(3-Chlorobenzylidene)-2-(benzo[*d*]thiazol-2-ylimino)thiazolidin-4-one 2i. Reaction time: 6 h. Yield: 81%; mp 208–210 °C (dioxane). TLC: eluent = toluene/dioxane/acetic acid 90/10/5. IR(KBr): ν = 3145 (N–H), 1708 (C=O), 1576 (N=C) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 13.03 (s, 1H, NH); 8.02 (d, 1H J = 7.5 H-4); 7.91 (d, 1H, J = 7.8 H-7); 7.70 -7.47 (m, 6H, H-5, H-2′, H-4′, H-5′, H-6′, CH); 7,37 (t, 1H, J = 7.5 H-6); $C_{17}H_{10}ClN_3OS_2$ (371.87): C, 54.91; H, 2.71; N, 11.30; S, 17.25. Found C, 54.51; H, 2.46; N, 11.03; S, 17.30%.

4.1.2.10. 5-(4-Chlorobenzylidene)-2-(benzo[*d*]thiazol-2-ylimino)thiazolidin-4-one 2h. Reaction time: 10 h. Yield: 45%; mp 248–250 °C (dioxane). TLC: eluent = toluene/dioxane/acetic acid 90/10/5. IR(KBr): ν = 3120 (N–H), 1699 (C=O), 1575 (N=C) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 12.98 (s, 1H, NH); 8.03 (d, 1H, J = 7.5 H-4); 7.92 (d, 1H, J = 7.8 H-7); 7.78 (s, 1H, CH); 7.74 (d, 2H, J = 8.7 H-2′, H-6′); 7.64 (d, 2H, J = 8.7 H-4′, H-5′); 7.51 (t, 1H, J = 7.5 H-5); 7.38 (t, 1H,J = 7.5 H-6). $C_{17}H_{10}ClN_3OS_2$ (371.87): C, 54.91; H, 2.71; N, 11.30; S, 17.25. Found C, 54.54; H, 2.85; N, 11.59; S, 17.52%.

4.1.2.11. 2-(Benzo[*d*]isothiazol-3-ylimino)-5-benzylidenethiazolidin-4-one 3a. Reaction time: 5 h. Yield: 89%; mp 210–211 °C (dioxane). TLC: eluent = methylene chloride/ethanol 95/5. IR(KBr): ν = 3072 (N–H), 1704 (C=O), 1592 (C=N) cm^{-1}.^1H NMR (DMSO-d_6 δ, ppm): 12.83 (s, 1H, NH); 8.20 (d, 1H, J = 8.1, H-4); 8.08 (d, 1H, J = 7.2, H-7); 7.75 (s, 1H, CH); 7.69–7.47 (m, 7H, H-5, H-6, H-2′, H-3′, H-4′, H-5′, H-6′). Anal. calcd for $C_{17}H_{11}N_3OS_2$ (337.42): C, 60.51; H, 3.29; N, 12.45; S, 19.01. Found C, 60.47; H, 3.32; N, 12.05; S, 18.78%.

4.1.2.12. 2-(Benzo[*d*]isothiazol-3-ylimino)-5-(4-hydroxybenzylidene)thiazolidin-4-one 3b. Reaction time: 7 h. Yield: 75%; mp 270–272 °C (dioxane). TLC: eluent = toluene/dioxane/acetic acid 90/10/5. IR(KBr): ν = 3336 (OH), 3207 (N–H), 1687 (C=O), 1589 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 12.63 (s, 1H, NH); 10.28 (s, 1H, OH); 8.19 (d, 1H, J = 8.1, H-4); 8.08 (d, 1H, J = 7.8, H-7); 7.67–7.62 (m, 2H, H-5, CH); 7.57–7.52 (m, 3H, H-6, H-2′, H-6′); 6.98 (d, 2H, J = 8.4, H-3′, H-5′). Anal. calcd for $C_{17}H_{11}N_3O_2S_2$ (353.42): C, 57.77; H, 3.14; N, 11.89; S, 18.15. Found C, 58.07; H, 3.43; N, 11.58; S, 17.95 %.

4.1.2.13. 2-(Benzo[*d*]isothiazol-3-ylimino)-5-(4-methoxybenzylidene)thiazolidin-4-one 3c. Reaction time: 5 h. Yield: 78%; mp 221–222 °C (dioxane). TLC: eluent = methylene chloride/ethanol 95/5. IR(KBr): ν = 3234 (N–H), 1716 (C=O), 1592 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 12.71 (s, 1H, NH); 8.17 (d, 1H, J = 8.1, H-4); 8.08 (d, 1H, J = 8.4, H-7); 7.70–7.62 (m, 4H, H-5, H-2′, H-6′, CH); 7.54 (t, 1H, J = 8.1, H-6); 7.14 (d, 2H, J = 8.7, H-3′, H-5′); 3.82 (s, 3H, CH_3O). Anal. calcd for $C_{18}H_{13}N_3O_2S_2$ (367.45): C, 58.84; H, 3.57; N, 11.44; S, 17.45. Found C, 58.83; H, 3.87; N, 11.06; S, 17.27%.

4.1.2.14. 2-(Benzo[*d*]isothiazol-3-ylimino)-5-(4-hydroxy-3- methoxybenzylidene)thiazolidin-4-one 3d. Reaction time: 20 h. Yield: 88%; mp 207–208 °C (dioxane). TLC: eluent = methylene chloride/ethanol 95/5. IR(KBr): ν = 3395 (OH), 3174 (NH), 1706 (C=O), 1579 (C=N)) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 12.31 (s, 1H, NH); 9.94 (s, 1H, OH); 8.18 (d, 1H, J = 8.1, H-4); 8.08 (d, 1H, J = 7.8, H-7); 7.67–7.62 (m, 2H, H-5, CH); 7.54 (t, 1H, J = 7.8, H-6); 7.28 (d, 1H, J = 1.8, H-2′); 7.16 (dd, 1H, J = 8.4, 2.1, H-6′); 7.01 (d, 1H, J = 8.4, H-5′); 3.83 (s, 3H, CH_3O). Anal. calcd for $C_{18}H_{13}N_3O_3S_2$ (383.45): C, 56.38; H, 3.42; N, 10.96; S, 16.73. Found C, 56.65; H, 3.62; N, 10.72; S, 16.37%.

4.1.2.15. 2-(Benzo[*d*]isothiazol-3-ylimino)-5-(2-nitrobenzylidene)thiazolidin-4-one 3e. Reaction time: 1 h. Yield: 78%; mp 208–210 °C (dioxane). TLC: eluent = methylene chloride/ethanol 95/5. IR(KBr): ν = 3190 (NH), 1705

(C=O), 1593 (C=N), 1514, 1332 (NO_2) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 12.92 (s,1H, NH); 8.22 (d, 1H,J = 8.4, H-3′); 8.15 (d, 1H, J = 8.1, H-6′); 8.06 (d, 1H, J = 7.8, H-4); 7.95–7.92 (m, 2H, CH, H-4′); 7.82 (d, 1H, J = 7.2, H-7); 7.73 (t, 1H, J = 7.6, H-5′); 7.65 (t, 1H, J = 7.2, H-5); 7.54 (t, 1H, J = 7.0, H-6). Anal. calcd for $C_{17}H_{10}N_4O_3S_2$ (382.42): C, 53.39; H, 2.64; N, 14.65; S, 16.77. Found C, 53.55; H, 2.83; N, 14.32; S, 16.61 %.

4.1.2.16. 2-(Benzo[*d*]isothiazol-3-ylimino)-5-(3-nitrobenzylidene)thiazolidin-4-one 3f. Reaction time: 1 h. Yield: 98%; mp 221–222 °C (dioxane). TLC: eluent = methylene chloride/ethanol 95/5. IR(KBr): v = 3135 (NH), 1700 (C=O), 1604 (C=N), 1527, 1353 (NO_2) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 12.96 (s,1H, NH); 8.52 (s, 1H, H-2′); 8.29 (dd, 1H, J = 8.4, 2.4, H-4′); 8.19 (d, 1H, J = 8.4, H-6′); 8.09–8.07 (m, 2H, H-4, H-7); 7.87–7.84 (m, 2H, CH, H-5′); 7.67 (t, 1H, J = 7.3, H-5); 7.55 (t, 1H, J = 7.2, H-6). Anal. calcd for $C_{17}H_{10}N_4O_3S_2$ (382.42): C, 53.39; H, 2.64; N, 14.65; S, 16.77. Found C, 53.52; H, 2.75; N, 14.46; S, 16.71%.

4.1.2.17. 2-(Benzo[*d*]isothiazol-3-ylimino)-5-(4-nitrobenzylidene)thiazolidin-4-one 3g. Reaction time: 2 h. Yield: 72%; mp 287–289 °C (dioxane). TLC: eluent = methylene chloride/ethanol 95/5. IR(KBr): v = 3114 (NH), 1708 (C=O), 1654 (C=N), 1511, 1344 (NO_2) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 12,94 (s,1H, NH); 8.37 (d, 2H, J = 8.7, H-3′, H-5′); 8.18 (d, 1H, J = 8.1, H-4); 8.07 (d, 1H, J = 8.1, H-7); 7.89 (d, 2H, J = 8.7, H-2′, H-6′); 7.79 (s, 1H, CH); 7.65 (t, 1H, J = 7.8, H-5); 7.54 (t, 1H, J = 7.8 H-6). Anal. calcd for $C_{17}H_{10}N_4O_3S_2$ (382.42): C, 53.39; H, 2.64; N, 14.65; S, 16.77. Found C, 53.51; H, 2.69; N, 14.70; S, 16.46 %.

4.1.2.18. 2-(Benzo[*d*]isothiazol-3-ylimino)-5-(2-chlorobenzylidene)thiazolidin-4-one 3h. Reaction time: 1 h. Yield: 92%; mp 214–215 °C (dioxane). TLC: eluent = methylene chloride/ethanol 95/5. IR(KBr): v = 3145 (NH), 1706 (C=O), 1590 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 12.93 (s, 1H, NH); 8.19 (d, 1H, J = 8.4, H-4); 8.08 (d, 1H, J = 8.1, H-7); 7.87 (s, 1H, CH); 7.72–7.47 (m, 6H, H-5, H-6, H-3′, H-4′, H-5′, H-6′). Anal. calcd for $C_{17}H_{10}ClN_3OS_2$ (371.87): C, 54.91; H, 2.71; N, 11.30; S, 17.25. Found C, 55.06; H, 2.74; N, 11.55; S, 17.29%.

4.1.2.19. 2-(Benzo[*d*]isothiazol-3-ylimino)-5-(3-chlorobenzylidene)thiazolidin-4-one 3i. Reaction time: 3 h. Yield: 85%; mp 227–228 °C (ethanol). TLC: eluent = methylene chloride/ethanol 95/5. IR(KBr): v = 3170 (NH), 1704 (C=O), 1610 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 12.86 (s, 1H, NH); 8.17 (d, 1H, J = 8.1, H-4); 8.06 (d, 1H, J = 8.1, H-7); 7.74–7.72 (m, 2H, H-2′, CH); 7.69–7.61 (m, 3H, H-5, H-5′, H-6′); 7.59–7.53 (m, 2H, H-6, H-4′). Anal. calcd for $C_{17}H_{10}ClN_3OS_2$ (371.87): C, 54.91; H, 2.71; N, 11.30; S, 17.25. Found C, 55.29; H, 2.85; N, 11.00; S, 17.18 %.

4.1.2.20. 2-(Benzo[*d*]isothiazol-3-ylimino)-5-(4-chlorobenzylidene)thiazolidin-4-one 3j. Reaction time: 9 h. Yield: 91%; mp 208–209 °C (dioxane). TLC: eluent = methylene chloride/ethanol 95/5. IR(KBr): v = 3120 (NH), 1718 (C=O), 1621(C=N) cm^{-1}. ^1H NMR (DMSO-d_6 δ, ppm): 12.85 (s, 1H, NH); 8.18 (d, 1H, J = 8.1, H-4); 8.07 (d, 1H, J = 8.4, H-7); 7.73–7.63 (m, 6H, H-5, H-2′, H-6′, H-3′, H-5′, CH); 7.55 (t, 1H, J = 7.8, 6). Anal. calcd for $C_{17}H_{10}ClN_3OS_2$ (371.87): C, 54.91; H, 2.71; N, 11.30; S, 17.25. Found C, 54.88; H, 2.92; N, 10.94; S, 17.16 %.

4.2. Microbiology

The antimicrobial activity was assayed in vitro by the two-fold broth dilution technique[18] against Gram positive bacteria (*Bacillus megaterium* BGSC 7A2, *Bacillus subtilis* ATCC 6633, *Bacillus thuringiensis* var. *kurstaki* BGSC 4D1, *Sarcina lutea* ATCC 9341, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228 and clinical isolates of methicillin-resistant *Staphylococcus aureus*, methicillin-resistant *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus agalactiae*, *Streptococcus faecalis*, *Streptococcus faecium* and *Streptococcus pyogenes*), Gram negative bacteria (*Escherichia coli* SPA 27 and a clinical isolate of *Haemophilus influenzae*), yeasts (*Candida tropicalis* ATCC 1369 and *Saccharomyces cerevisiae* ATCC 9763) and moulds (*Aspergillus niger* ATCC 6275). The minimal inhibitory concentrations (MIC, μg/mL) were defined as the lowest concentrations of compound that completely inhibited the growth of each strain. All compounds, dissolved in dimethylsulfoxide, were added to culture media (Haemophilus Test Medium for *H. influenzae*, Tryptose Phosphate Broth for *S. pyogenes*, Mueller Hinton Broth for other bacteria and Sabouraud Liquid Medium for fungi) to obtain final concentrations ranging from 100 μg/mL to 0.0015 μg/mL. The amount of dimethylsulfoxide never exceeded 1% v/v. Inocula consisted of 5.10^4 bacteria/mL and 1.10^3 fungi/mL. The MICs were read after incubation at 37 °C for 24 h (bacteria) and at 30 °C for 48 h (fungi). Media and media with 1% v/v dimethylsulfoxide were employed as growth controls. Ampicillin and miconazole were used as reference antibacterial and antifungal drugs, respectively.

To detect the type of antimicrobial activity, subcultures were performed by transferring 100 μL of each mixture remaining clear in 1 mL of fresh medium. The minimal bactericidal concentrations (MBC, μg/mL) and the minimal fungicidal concentrations (MFC, μg/mL) were read after incubation at 37 °C for 24 h and at 30 °C for 48 h, respectively.

All experiments were performed in triplicate and repeated three times.

4.3. Computational methods

The compounds were processed through the DEREK software,[19] which predicts the probability of toxicity for a number of endpoints.

For the QSAR analysis, a large number of molecular descriptors were calculated using TSAR,[20] HYBOT[21] and ADMEWORKS Predictor[22] software. The ADMEWORKS Predictor software includes the ADAPT software developed by Jurs[23] [ADAPT].

The biological endpoint values were used in logarithmic form, both because QSARs are considered to be free energy relationships, and because logarithmic values tend to be less skewed. For the QSAR calculations the concentration results (MIC) were converted from μg/mL to mmol/L.

Using the MOBYDIGS software,[24] we removed one of each pair of descriptors with high collinearity, and used the software's genetic algorithm (GA) procedure to select the best 10 QSAR models for each endpoint, from the remaining 256 descriptors.

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