European Journal of Medicinal Chemistry 167 (2019) 200–210

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

2,6-Disubstituted imidazo[2,1-*b*][1,3,4]thiadiazole derivatives as potent staphylococcal biofilm inhibitors

Stella Cascioferro [a], Barbara Parrino [a], Giovanna Li Petri [a], Maria Grazia Cusimano [a], Domenico Schillaci [a], Veronica Di Sarno [b], Simona Musella [b], Elisa Giovannetti [c, d], Girolamo Cirrincione [a], Patrizia Diana [a, *]

[a] *Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Via Archirafi 32, 90123, Palermo, Italy*
[b] *Dipartimento di Farmacia, Università degli Studi di Salerno, Via G. Paolo II 132, 84084, Fisciano, Salerno, Italy*
[c] *Department of Medical Oncology, VU University Medical Center, Cancer Center Amsterdam, De Boelelaan 1117, 1081HV, Amsterdam, the Netherlands*
[d] *Cancer Pharmacology Lab, AIRC Start-Up Unit, University of Pisa and Fondazione Pisana per la Scienza, via Via Ferruccio Giovannini 13, 56017, San Giuliano Terme, Pisa, Italy*

ARTICLE INFO

Article history:
Received 11 December 2018
Received in revised form
1 February 2019
Accepted 2 February 2019
Available online 10 February 2019

Keywords:
Staphylococcal biofilm inhibitors
imidazo[2,1-*b*][1,3,4]thiadiazole derivatives
Anti-virulence agents
Anti-Biofilm agents

ABSTRACT

A class of 36 new 2-(6-phenylimidazo[2,-1-*b*][1,3,4]thiadiazol-2-yl)-1*H*-indoles was efficiently synthesized and evaluated for their anti-biofilm properties against the Gram-positive bacterial reference strains *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 12228, and the Gram-negative strains *Pseudomonas aeruginosa* ATCC 15442 and *Escherichia coli* ATCC 25922. Many of these new compounds, were able to inhibit biofilm formation of the tested staphylococcal strains showing BIC_{50} lower than 10 μg/ml. In particular, derivatives **9c** and **9h** showed remarkable anti-biofilm activity against *S. aureus* ATCC 25923 with BIC_{50} values of 0.5 and 0.8 μg/ml, respectively, whereas compound **9aa** was the most potent against *S. aureus* ATCC 6538, with a BIC_{50} of 0.3 μg/ml. Remarkably, these compounds showed effects in the early stages of the biofilm formation without affecting the mature biofilm of the same strains and the viability of the planktonic form. Their ability in counteracting a virulence factor (biofilm formation) without interfering with the bacterial growth in the free life form make them novel valuable anti-virulence agents.

1. Introduction

Antibiotic resistance, caused by the overuse/misuse of antibiotics and by the great evolutionary capacity of microorganisms, is currently considered a serious global threat.

Common pathogens including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species, known as ESKAPE pathogens (e.g., capable of 'escaping' the biocidal action of antibiotics), are the leading cause of severe nosocomial infections which became resistant to various antibiotic therapies. As a counteracting measure, in the last decade, many efforts have been made for the development of new agents that target the virulence mechanisms of important pathogens without affecting their viability. These new therapeutic strategies aim indeed at imposing limited selective pressure in promoting the development of the antibiotic-resistance [1–3].

According to current estimates by the National Institute of Health, a percentage ranging from 65 to 80% of all bacterial infections are biofilm-mediated [4]. The ability to grow as a biofilm is considered one of the main natural resistance mechanisms developed by pathogens as well as one of the most relevant virulence factor. This is indeed the main cause of nosocomial chronic infections which are difficult to treat with conventional antibiotics, including periodontitis, pneumonia in cystic fibrosis patients, and numerous infections associated with indwelling devices such as catheters, heart valves, and prostheses [5,6]. The biofilm is a stratified bacterial community that grows on a biological or artificial surface, characterized by a multifactorial tolerance to antibiotics. In addition to limiting the penetration of antibiotics, biofilms, contain, in the deepest layers, bacterial subpopulations (dormant

* Corresponding author.
E-mail address: patrizia.diana@unipa.it (P. Diana).

https://doi.org/10.1016/j.ejmech.2019.02.007
0223-5234/

cells) with low metabolic activity and proliferation rate that are intrinsically resistant to conventional antibiotics. In this bacterial community, single cells are embedded in a self-made polymeric matrix essentially composed by exopolysaccharides or other extracellular polymeric molecules, including extracellular DNA, amyloid fibers, etc., as well as molecules originating from the host, such as mucus and DNA.

Conventional antibiotic therapies effective on planktonic cells are usually inactive against biofilms. In the last few years, several attempts have been made to obtain new molecules able to interfere with the biofilm formation suitable for the treatment of biofilm-associated infections [7–12].

Currently the main strategies to counteract biofilms involve prevention of their formation or the dispersing of mature biofilm. Inhibitors of biofilm formation are often compounds able to inhibit the microbial attachment to surfaces, interfering with the bacterial adhesion, which is considered the initial step in bacterial pathogenesis. Other potential mechanisms of action for potential anti-biofilm therapeutics consist in: i) regulating the quorum sensing (QS) system which is the bacterial cell-to-cell signaling responsible for the coordination of many virulence factors, including biofilm formation; ii) interfering with regulatory mechanisms such as nucleotide second messenger signaling systems, and (iii) disrupting the biofilm structure [13].

Indole compounds are widely described for their therapeutical potential as anti-inflammatory, analgesic [14], antiviral [15], anti-cancer [16–19] and antibacterial agents [20].

Indole is produced by more than 85 species of pathogens as bacterial intercellular signal, which plays a key role in modulating *E. coli* biofilm formation and *P. aeruginosa* virulence by repressing motililty, chemotaxis and bacterial adhesion [13,21–23].

Lee et al. described the activity of indole-3-acetic acid, 3,3′-methylene bisindole, indole-3-propioninc acid, indole-3-carbinol, indole-3-carboxyaldehyde and 3-indolylacetonitrile in inhibiting *E. coli* O157:H7 and *P. aeruginosa* biofilm formation without affecting microbial growth [24].

Moreover the imidazo[2,1-*b*] [1,3,4]thiadiazole ring system has been recognized as a privileged scaffold for obtaining molecules with a broad spectrum of biological activities, such as anticancer [25], antioxidant [26], antitubercolar [27], anticonvulsivant [28], analgesic [29], and antibacterial [30,31].

Despite the numerous therapeutic properties described for the imidazo[2,1-*b*] [1,3,4]thiadiazole derivatives, the antibiofilm activity of this class of compounds has never been described.

However, the isosters triazolothiazole compounds **1**–**3** (Fig. 1) were reported by Zhang et al. [32] as potent inhibitors of Sortase A (SrtA), a Gram-positive transpeptidase involved in the process of bacterial adhesion and, therefore, closely related to the biofilm formation process [33,34]. Notably, compound **3** showed a significant *in vivo* anti-infective activity in preventing *S. aureus* bloodstream infections.

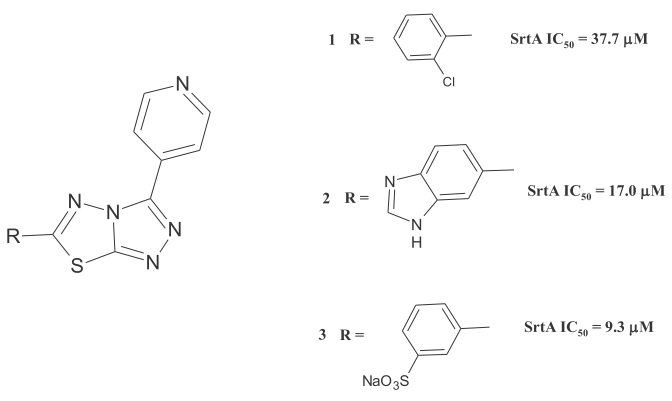


Fig. 1. Chemical structures of triazolothiazole compounds **1**–**3**.

Our previous researches focused on nitrogen heterocyclic systems to counteract the global threat of the antibiotic resistance [35–37], and we then develop a special interest towards indole moiety [38–41]. Therefore, considering the interesting therapeutic potentials of the imidazo[2,1-*b*] [1,3,4]thiadiazole scaffold, herein we report the synthesis of a new series of 2-(6-phenylimidazo[2,-1-*b*] [1,3,4]thiadiazol-2-yl)-1*H*-indole derivatives. These novel compounds were assayed for their preventive effect on biofilm formation of the Gram-positive bacterial reference strains *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 12228, and of the Gram-negative strains *Pseudomonas aeruginosa* ATCC 15442 and *Escherichia coli* ATCC 25922.

2. Chemistry

A series of 36 new imidazothiadiazole derivatives **9** and **10** was efficiently synthesized as described in Scheme 1. The 1*H*-indole-3-carbonitrile **5a** was commercially available, whereas carbonitriles **5b,c** were obtained by reaction of the corresponding 1*H*-indole **4** with the chlorosulfonyl isocyanate (CSI) in anhydrous acetonitrile at 0 °C in yield 98–100%. The methylation of derivatives **5** with dimethyl carbonate in anhydrous DMF under reflux at 130 °C, afforded the corresponding 1-methyl-1*H*-indole-3-carbonitrile **6** (yield 98%). The key intermediates **7a-f** were prepared by reaction of carbonitriles **5** and **6** thus obtained, with thiosemicarbazide in trifluoroacetic acid (TFA) at 60 °C for 3 h (yield 98–100%).

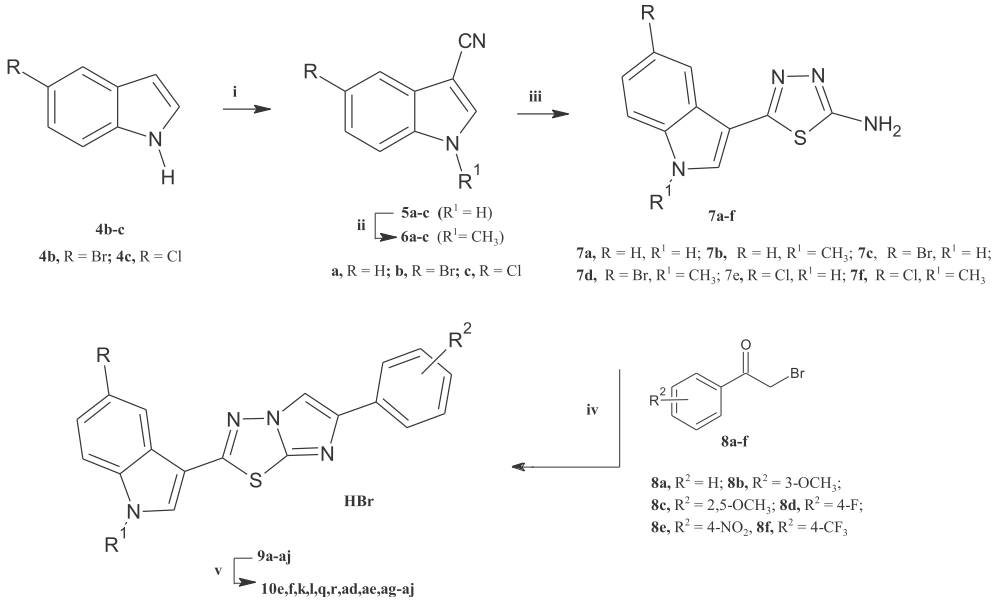
Finally, the reaction between the appropriate 5-(1*H*-indol-3-yl)-1,3,4-thiadiazol-2-amine **7a-f** and the suitable α-bromoacetyl compounds **8a-f** in ethanol under reflux for 24 h gave the desired new 2-(6-phenylimidazo[2,-1-*b*] [1,3,4]thiadiazol-2-yl)-1*H*-indoles **9a-aj** as hydrobromide salts. Compounds **9e**,**f**,**k**,**l**,**q**,**r**,**ad**,**ae**,**ag-aj**. were treated with saturated aqueous $NaHCO_3$ solution for obtaining the corresponding free bases **10** which were purified by silica gel column chromatography eluting by dichloromethane:ethyl acetate, 1:1 (42–55%) (Table 1).

Reagents and conditions: i) CH_3CN, CSI, 0 °C, 2 h, then DMF, 0 °C, 1.5 h (98–100%); ii) DMF, $(CH_3O)_2CO$, K_2CO_3, 130 °C, 3.5 h; iii) trifluoroacetic acid, thiosemicarbazide, 60 °C, 3.5 h (98%); iv) anhydrous ethanol, 2-bromo-1-phenylethan-1-one **8**, reflux, 24 h (42–55%); v) $NaHCO_3$ saturated aqueous solution.

3. Results and discussion

3.1. Inhibition of biofilm formation

All the synthesized compounds **9a-d,g-j,m-p,s-ac,af** (hydrobromide salts) and **10e**,**f**,**k**,**l**,**q**,**r**,**ad**,**ae**,**ag-aj** (free bases) were primarily assayed *in vitro* for their antibacterial activity against the planktonic form of the Gram-positive bacterial reference strains *S. aureus* ATCC 25923, *S. aureus* ATCC 6538 and *S. epidermidis* ATCC 12228, and of the Gram-negative strains *P. aeruginosa* ATCC 15442 and *E. coli* ATCC 25922. and the minimum inhibitory concentration (MIC) of the tested compounds was evaluated. The obtained results highlighted no interference by the imidazo[2,1-*b*] [1,3,4]thiadiazoles **9** and **10** on the microbial planktonic growth (MIC > 100 μg/mL). This result was desirable from the perspective of obtaining derivatives with an anti-virulence profile, namely compounds able to target key virulence factors rather than killing or inhibiting the growth of pathogens, which is advantageous for imposing limited selective pressure in promoting the development of the antibiotic-resistance mechanisms.



Scheme 1. Synthesis of new 2-(6-phenylimidazo[2,1-*b*] [1,3,4]thiadiazol-2-yl)-1*H*-indoles **9a-aj**.

All the synthesized compounds **9** and **10** were then tested *in vitro* in order to evaluate their ability in inhibiting biofilm formation of the five tested strains. Compounds **9m,n,p,v,y,aj** and **10f,l,** showed no effect on the biofilm formation in the tested strains. For all the other synthesized derivatives the biofilm inhibitory concentration (BIC_{50}) values, that is the concentration at which the percentage of inhibition of biofilm formation is equal to 50% compared to the untreated growth control, were determined and reported in Table 2.

All compounds were able to interfere with the bacterial biofilm formation of the tested Gram-positive pathogens in a dose-dependent manner eliciting in many cases BIC_{50} values lower than 10 μg/ml. Interestingly, compounds **9c, 9h** and **9aa,** bearing one or two methoxy groups on the phenyl ring, displayed the best anti-biofilm activity against all tested Gram-positive strains with BIC_{50} values ranging from 0.5 to 18.5 μg/ml. In particular, derivatives **9c** and **9h** showed remarkable anti-biofilm activity against *S. aureus* ATCC 25923 with BIC_{50} values of 0.5 and 0.8 μg/ml, respectively, whereas compound **9aa** was the most potent against *S. aureus* ATCC 6538 with a BIC_{50} of 0.3 μg/ml.

Noteworthy compounds **10k** and **9w,** which bring a nitro substituent in para position of the phenyl ring, significantly inhibited biofilm formation in all Gram-positive and Gram-negative tested strains showing BIC_{50} between 1.4 and 38.4 μg/ml.

These results highlighted the influence of the substituents in the phenyl ring on the ability of this class of compounds in interfering with the biofilm formation. The presence of electron-donating methoxy groups was indeed advantageous for the anti-biofilm activity against Gram-positive pathogens, while the substitution on the phenyl ring with electron-withdrawing nitro groups improved the inhibitory activity against Gram-negative pathogens.

Conversely, the structural modifications made at the indole nucleus, such as the introduction of a halogen atom at the 5 position or the methylation of the nitrogen atom, did not significantly influence the anti-biofilm activity of the compounds.

The minimal concentrations required to inhibit 90% of biofilm formation (Table 3), as well as the concentration vs inhibition graph (Fig. 2) were evaluated for the most active derivatives **9aa**, **9c** and **9h.**

The new derivatives **9c, 9h** and **9aa** showed higher potency in inhibiting staphylococcal biofilm formation compared to the other indole derivatives so far described and elicited activity comparable to the most potent anti-biofilm compounds reported in literature [13].

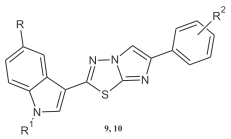
Respect to the previous series of anti-biofilm compounds synthesized by us [35–37], a significant improvement in the inhibition of biofilm formation was observed. Among them, only the thiazole nortopsentin derivatives recently described [35] showed analogous inhibitory activity against staphylococcal biofilm with BIC_{50}, in some cases, in the low micromolar range, highlighting the importance of the indole scaffold for the anti-biofilm activity.

Compounds **9c**, **9h**, **9i**, **9w**, **9aa** and **10k**, which were the most potent against the *S. aureus* strains, were also tested with the aim to evaluate their dispersal activity against the 24 h preformed biofilm of the same strains. Among the tested compounds, only derivative **9c** showed a weak activity eliciting an IC_{50} value of 142.5 μg/ml against *S. aureus* ATCC 25923.

These results suggested a mechanism of anti-biofilm activity related to the interference with adhesion or regulatory mechanisms involved in bacterial communication systems characterizing the first steps of biofilm formation, rather than an ability to disrupt mature biofilms.

The selectivity showed by most of our compounds towards Gram-positive pathogens and their activity in the first stages of the biofilm formation without interferences on the microbial viability and on the preformed biofilm led us to hypothesize the inhibition of SrtA as possible mechanism of action [33]. To validate this hypothesis, compounds **9c, 9h, 9k** and **9aa,** which showed the highest anti-biofilm activity and the best selectivity towards the Gram-positive strains, were selected for evaluating their inhibitory activity against *S. aureus* SrtA. However, no compound proved to be effective at the maximum tested concentration of 100 μM against the transpeptidase. We therefore speculate that many other targets can be involved in the anti-biofilm activity of this class of compounds, including autoinducing peptides (AIPs), autoinducer-2 (AI-2), bacterial second messengers, such as c-di-GMP and c-di-AMP, and indole pathway [13]. In particular, indole plays a key role in the communication system employed by the bacteria to coordinate many processes involved in the antibiotic resistance including biofilm formation, bacterial virulence, motility and dormant cell

Table 1
New 2-(6-phenylimidazo[2,1-*b*] [1,3,4]thiadiazol-2-yl)-1*H*-indole derivatives **9** (hydrobromide salts) and **10** (free bases).



Comp	R	R^1	R^2	Yield(%)
9a	H	H	H	47
9b	H	H	3-OCH_3	43
9c	H	H	2,5-OCH_3	42
9d	H	H	4-F	42
9g	H	CH_3	H	50
9h	H	CH_3	3-OCH_3	52
9i	H	CH_3	2,5-OCH_3	48
9j	H	CH_3	4-F	50
9m	Br	H	H	50
9n	Br	H	3-OCH_3	50
9o	Br	H	2,5-OCH_3	43
9p	Br	H	4-F	42
9s	Br	CH_3	H	52
9t	Br	CH_3	3-OCH_3	45
9u	Br	CH_3	2,5-OCH_3	53
9v	Br	CH_3	4-F	48
9w	Br	CH_3	4-NO_2	46
9x	Br	CH_3	4-CF_3	42
9y	Cl	H	H	45
9z	Cl	H	3-OCH_3	46
9aa	Cl	H	2,5-OCH_3	46
9ab	Cl	H	4-F	45
9ac	Cl	H	4-NO_2	42
9af	Cl	CH_3	3-OCH_3	50
10e	H	H	4-NO_2	52
10f	H	H	4-CF_3	50
10k	H	CH_3	4-NO_2	52
10l	H	CH_3	4-CF_3	42
10q	Br	H	4-NO_2	42
10r	Br	H	4-CF_3	45
10ad	Cl	H	4-CF_3	46
10ae	Cl	CH_3	H	42
10ag	Cl	CH_3	2,5-OCH_3	55
10ah	Cl	CH_3	4-F	42
10ai	Cl	CH_3	4-NO_2	48
10aj	Cl	CH_3	4-CF_3	43

formation. It was reported as indole-containing small molecules are able to decrease in *S. aureus* the production of staphyloxanthin, which is a virulence factor responsible for the oxidant and neutrophil resistance of the pathogen. Additionally, in *S. aureus,* the interference with the indole pathway inhibits the hemolytic activity of the bacterium against human red blood cells.

Indole-signaling pathway is currently considered a valuable target to counteract bacterial virulence and biofilm formation. A recent study reported many indole-containing small molecules able to inhibit biofilm formation by interfering with the bacterial indole signalling [42].

Given the presence of the indole nucleus in these derivatives and considered that the anti-biofilm properties of most indole derivatives were due to the interference with the indole pathway, we hypothesize that our new compounds might act by this mechanism.

4. Conclusions

A library of 36 new 2-(6-phenylimidazo[2,-1-*b*] [1,3,4]thiadiazol-2-yl)-1*H*-indoles **9** and **10** has been efficiently synthesized and evaluated for its anti-biofilm properties.

Despite the numerous biological properties described for the imidazo[2,1-*b*] [1,3,4]thiadiazole scaffold, this is the first time that its anti-biofilm activity was reported. Among the synthesized compounds many derivatives were able to inhibit the biofilm formation of the tested staphylococcal strains showing BIC_{50} lower than 10 μg/ml, and in three cases (**9c**, **9h** and **9aa**) lower than 1 μg/ml.

Remarkably, compounds **9c**, **9h**, **9i**, **9w, 9aa** and **10k** showed the typical behaviour of anti-virulence agents, inhibiting the formation of the bacterial biofilm, which is considered one of the most important bacterial virulence factor, especially in the case of microorganisms once considered harmless such as *S. epidermidis*.

The anti-virulence strategy, aimed to counteract virulence factors without interfering with the microbial growth, has attracted increasing interest in the fight against the antibiotic resistance. Therefore the derivatives **9c**, **9h** and **9aa** could be considered attractive lead compounds for developing a new class of potent inhibitors of staphylococcal biofilm formation. These innovative antibiofilm strategies warrant further studies, in order to obtain effective approaches that will be included in the therapeutic arsenals for use against difficult-to-treat infections, such as chronic and medical devices associated infections.

5. Experimental

5.1. Chemistry

All melting points were taken on a Büchi-Tottoly capillary apparatus and are uncorrected. IR spectra were determined in bromoform with a Shimadzu FT/IR 8400S spectrophotometer. ^1H and ^{13}C NMR spectra were measured at 200 and 50.0 MHz, respectively, in DMSO-d_6 solution, using a Bruker Avance II series 200 MHz spectrometer. Column chromatography was performed with Merck silica gel 230–400 mesh ASTM or with Büchi Sepacor chromatography module (prepacked cartridge system). Elemental analyses (C, H, N) were within ±0.4% of theoretical values and were performed with a VARIO EL III elemental analyzer. Purity of all the tested compounds was greater than 95%, determined by HPLC (Agilent 1100 Series).

5.1.1. General procedure for the synthesis of 1H-indole-3-carbonitriles (*5b-c)*

To a solution of the indole **4b,c** (5.10 mmol) in anhydrous acetonitrile (4.5 ml), chlorosulfonyl isocyanate (CSI) (0.44 ml, 5.10 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 2 h. Anhydrous dimethylformamide (DMF) (2.8 ml, 36.39 mmol) was slowly added and the mixture was maintained under stirring at 0 °C for 1.5 h. The reaction mixture was poured into crushed ice and the precipitate was filtered off (yields 98–100%).

Analytical and spectroscopic data for compounds **5b,c** are in agreement with those previously reported [43].

5.1.2. General procedure for the synthesis of 1-methylindole-3-carbonitriles (*6a-c)*

A mixture of the suitable 3-cyanoindole **5a-c** (7.03 mmol), 0.5 g of K_2CO_3, anhydrous DMF (10 ml) and dimethyl carbonate (1.8 ml, 21.4 mmol) was heated at 130 °C for 3.5 h. Then the reaction mixture was cooled to 3 °C and water and ice (25 ml) was slowly added under stirring. The oily suspension thus obtained was extracted with diethyl ether (3 × 10 ml) and the organic layer was washed with water and brine, dried over anhydrous Na_2SO_4 and evaporated under vacuum to obtain the 3-cyano-1-methylindole **6a-c** in excellent yields.

Analytical and spectroscopic data are in accordance to those reported in literature [44].

Table 2
Inhibition of biofilm formation, BIC_{50}, μg/ml (μM).

Comp	*S. aureus* ATCC 25923	*S. aureus* ATCC 6538	*S. epidermidis* ATCC 12228	*P. aeruginosa* 15442	*E. coli* 25922
9a	14.3 (35.9)	18.5 (46.5)	23.5 (59.1)	n.s.	n.s.
9b	34.3 (80.2)	16.1 (37.0)	14.3 (33.4)	34.3 (80.2)	29.8 (69.7)
9c	0.5 (1.2)	8.5 (18.5)	13.7 (29.9)	n.s.	n.s.
9d	6.5 (15.6)	5.6 (13.4)	12.8 (30.8)	19.7 (47.4)	n.s.
9g	9.5 (23)	7.8 (18.9)	19.7 (47.9)	n.s.	n.s.
9h	0.8 (1.8)	12.4 (28.0)	18.5 (41.9)	n.s.	n.s.
9i	11.5 (24.3)	1.7 (3.6)	11.8 (25.0)	42.9 (91.0)	n.s.
9j	3.7 (8.6)	7.7 (17.9)	29.6 (68.9)	n.s.	n.s.
9o	14.2 (26.4)	8.9 (16.5)	19.4 (36.1)	n.s.	n.s.
9s	n.s.	n.s.	10.6 (21.6)	n.s.	n.s.
9t	8.5 (16.0)	6.5 (12.4)	33.2 (63.8)	13.7 (26.3)	n.s.
9u	50 (90.8)	4.9 (8.9)	28.8 (52.3)	15.7 (28.5)	n.s.
9w	11.2 (20.9)	1.7 (3.1)	38.4 (71.7)	20.7 (38.6)	10.5 (19.6)
9z	14.6 (31.6)	7.0 (15.1)	15.1 (32.7)	13.4 (29.0)	n.s.
9aa	11.9 (24.2)	0.3 (0.5)	9.3 (18.9)	n.s.	45 (91.5)
9ab	23.1 (51.3)	10.9 (24.2)	45.3 (100.7)	n.s.	n.s.
9ac	26.4 (55.3)	17.1 (35.8)	13.6 (28.5)	n.s.	n.s.
9af	n.s.	n.s.	11.4 (23.9)	n.s.	n.s.
10e	17.2 (47.5)	7.3 (20.2)	14.9 (41.2)	n.s.	n.s.
10k	7.9 (21.0)	1.4 (3.0)	7.9 (21.0)	11.3 (30.1)	12.9 (34.3)
10q	12.5 (23.9)	14.1 (27.0)	9.9 (19.1)	22.6 (43.3)	n.s.
10r	7.8 (16.8)	6.2 (13.3)	8.9 (19.2)	n.s.	n.s.
10ad	4.1 (9.7)	5.3 (12.6)	10.3 (24.5)	n.s.	n.s.
10ae	n.s.	n.s.	16.3 (36.5)	n.s.	n.s.
10ag	82.5 (163.0)	35.1 (69.4)	4.1 (8.1)	n.s.	n.s.
10ah	n.s.	23.4 (50.4)	7.9 (17.0)	n.s.	n.s.
10ai	n.s.	16.2 (33.0)	38.3 (78.0)	n.s.	n.s.

n.s. not significant because lower than 15% of inhibition percentage at the screening concentration of 100 μg/ml.

Table 3
Inhibition of biofilm formation expressed as BIC_{90}.

Compounds	BIC_{90}[μg/ml]
9c (*S.aureus* 25923)	36.5
9h (*S.aureus* 25923)	23.3
9aa (*S.aureus* 6538)	16.2

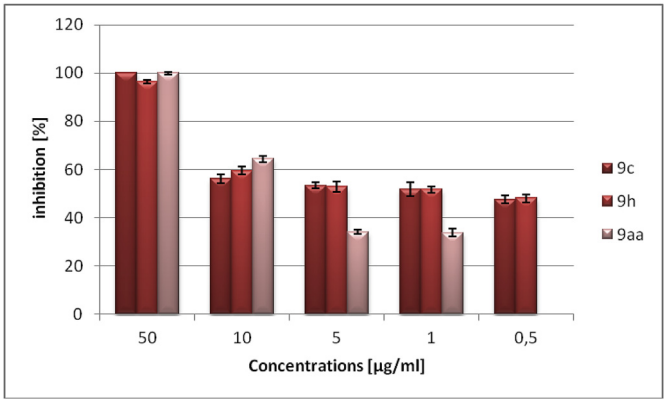


Fig. 2. Concentration versus inhibition percentage graph, **9c** and **9h** towards *S. aureus* ATCC 25923, and **9aa** towards *S. aureus* ATCC 6538.

5.1.3. General procedure for the synthesis of 5-(1H-indol-3-yl)-1,3,4-thiadiazol-2-amines (**7a-f**)

A solution of the appropriate 1*H*-indole-3-carbonitriles **5a-c** or 1-methylindole-3-carbonitriles **6a-c** (5 mmol) and thiosemicarbazide (5 mmol) in trifluoroacetic acid (5 ml) was heated at 60 °C for 3.5 h. The reaction mixture was then poured into ice and slowly neutralized with $NaHCO_3$ saturated solution. The obtained precipitate was filtered off, washed with water, cyclohexane and diethyl ether.

*5.1.3.1. 5-(1H-Indol-3-yl)-1,3,4-thiadiazol-2-amine (**7a**).* Light yellow solid, yield: 98%, m.p. 210–211 °C, IR cm^{-1}: 3609 (NH), 3461-3210 (NH_2); ^{1}HNMR DMSO-d_6 (ppm): 7.15–7.24 (4H, m, Ar-H, NH_2), 7.45 (1H, d, $J = 6.75$ Hz, Ar-H), 7.85 (1H, d, $J = 2.62$ Hz, Ar-H), 8.11 (1H, d, $J = 6.98$ Hz, Ar-H), 11.64 (1H, s, NH). ^{13}C NMR DMSO-d_6 (ppm): 107.2 (s), 111.9 (d), 120.4 (d), 120.6 (d), 122.4 (d), 124.1 (s), 126.6 (d), 136.4 (s), 152.3 (s), 165.8 (s). Anal. Calcd for $C_{10}H_8N_4S$ (MW 216.26): C, 55.54%; H, 3.73%; N, 25.91%. Found: C, 55.71%; H, 3.68%; N, 25.75%.

*5.1.3.2. 5-(1-Methyl-1H-indol-3-yl)-1,3,4-thiadiazol-2-amine (**7b**).* Light yellow solid, yield: 98%, m.p. 124–125 °C, IR cm^{-1}: 3612 (NH), 3478-3228 (NH_2), ^{1}HNMR DMSO-d_6 (ppm): 3.85 (3H, s, CH_3), 7.23–7.35 (2H, m, Ar-H), 7.55 (1H, d, $J = 7.41$ Hz, Ar-H), 8.06 (2H, d, $J = 6.05$ Hz, Ar-H), 8.57 (2H, bs, NH_2), ^{13}C NMR DMSO-d_6 (ppm): 33.3 (q), 107.5 (s), 110.6 (d), 120.6 (d), 121.2 (d), 122.8 (d), 124.2 (s), 131.8 (d), 136.2 (s), 152.4 (s), 166.8 (s). Anal. Calcd for $C_{11}H_{10}N_4S$ (MW 230.29): C, 57.37%; H, 4.38%; N, 24.33%. Found: C, 57.48%; H, 4.42%; N, 24.51%.

*5.1.3.3. 5-(5-Bromo-1H-indol-3-yl)-1,3,4-thiadiazol-2-amine (**7c**).* Yellow solid, yield: 92%, m.p. 232–233 °C. IR cm^{-1}: 3445 (NH), 4147 (NH_2). ^{1}HNMR (200 MHz, DMSO-d_6) δ: 7.19–7.46 (4H, m, ArH, NH_2), 7.91 (1H, s, Ar-H), 8.30 (1H, s, Ar-H), 11.83 (1H, bs, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 107.0 (s), 112.9 (s), 113.9 (d), 123.0 (d), 124.9 (d), 125.8 (s), 127.9 (d), 135.1 (s), 151.8 (s), 165.9 (s). Anal. Calcd for $C_{10}H_7BrN_4S$ (MW 295.16): C, 40.69%; H, 2.39%; N, 8.98%. Found: C, 40.72%; H, 2.36%; N, 8.95%.

*5.1.3.4. 5-(5-Bromo-1-methyl-1H-indol-3-yl)-1,3,4-thiadiazol-2-amine (**7d**).* Yellow solid, yield: 98%, m.p. 174–175 °C, IR cm^{-1}: 3558 (NH_2), ^{1}HNMR DMSO-d_6 (ppm): 3.84 (3H, s, CH_3), 7.51 (2H, dd, $J = 1.92$, 8.75 Hz, Ar-H), 8.08 (3H, m, Ar-H, NH_2), 8.23 (1H, s, Ar-H). ^{13}C NMR DMSO-d_6 (ppm): 32.9 (q), 105.4 (s), 112.7 (d), 113.5 (s), 123.0 (d), 125.1 (d), 125.9 (s), 132.3 (d), 135.7 (s), 151.3 (s), 166.2 (s).

Anal. Calcd for $C_{11}H_9BrN_4S$ (MW 309.19): C, 42.73%; H, 2.93%; N, 18.12%. Found: C, 42.82%; H, 3.01%; N, 18.24%.

*5.1.3.5. 5-(5-Chloro-1H-indol-3-yl)-1,3,4-thiadiazol-2-amine (**7e**).* Orange solid, yield: 98%, m.p. 234–235 °C, IR cm^{-1}: 3564 (NH), 3255 (NH_2), ^{1}HNMR DMSO-d_6 (ppm): 7.19–7.50 (4H, m, Ar-H, NH_2), 7.98 (1H, s, Ar-H), 8.14 (1H, s, Ar-H), 11.87 (1H, s, NH). ^{13}C NMR DMSO-d_6 (ppm): 106.9 (s), 113.6 (d), 119.9 (d), 122.5 (d), 125.0 (s), 125.1 (s), 128.4 (d), 134.9 (s), 151.8 (s), 166.0 (s). Anal. Calcd for $C_{10}H_7ClN_4S$ (MW 250.70): C, 47.91%; H, 2.81%; N, 22.35%. Found: C, 47.75%; H, 2.92%; N, 22.54%.

*5.1.3.6. 5-(5-Chloro-1-methyl-1H-indol-3-yl)-1,3,4-thiadiazol-2-amine (**7f**).* Yellow solid, yield: 100%, m.p. 95–96 °C, IR cm^{-1}: 3609 (NH_2), ^{1}HNMR DMSO-d_6 (ppm): 3.84 (3H, s, CH_3), 7.29 (1H, d, J = 8.77, Ar-H), 7.58 (1H, d, J = 8.76, Ar-H), 8.08 (4H, Ar-H, NH_2). ^{13}C NMR DMSO-d_6 (ppm): 33.0 (q), 105.2 (s), 112.3 (d), 119.9 (d), 122.7 (d), 125.2 (s), 125.7 (s), 132.7 (d), 135.5 (s), 166.4 (s). Anal. Calcd for $C_{11}H_9ClN_4S$ (MW 264.74): C, 49.91%; H, 3.43%; N, 21.16%. Found: C, 50.03%; H, 3.68%; N, 21.32%.

*5.1.4. General procedure for the synthesis of 3-(6-Phenylimidazo [2,1-b][1,3,4]thiadiazol-2-yl)-1H-indoles derivatives **9a-d,g-j,m-p,s-ac,af** and **10e,f,k,l,q,r,ad,ae,ag,ah,ai,aj***

A solution of the suitable 5-(1*H*-indol-3-yl)-1,3,4-thiadiazol-2-amine **7a-f** (0.92 mmol) and the appropriate 2-bromo-1-phenylethanone **8a-f** (0.92 mmol) in 40 ml of anhydrous ethanol was heated under reflux for 24 h. Upon cooling, the corresponding hydrobrobide was filtered off and washed with ethanol. Derivatives **9a-d,g-j,m-p,s-ac,af** were isolated as pure compounds and were characterized without further purifications. Instead, in the case of compounds **9e**,**f**,**k**,**l**,**q**,**r**,**ad**,**ae**,**ag**-**aj**. it was necessary the treatment with saturated aqueous $NaHCO_3$ solution for obtaining the corresponding free base **10** which was purified by silica gel column chromatography eluting by dichloromethane:ethyl acetate, 1:1. Derivatives **9k,q,w,ac,ai,v** were characterized only by ^{1}HNMR spectra due to their poor solubility.

*5.1.4.1. 3-(6-Phenylimidazo[2,1-b][1,3,4]thiadiazol-2-yl)-1H-indole hydrobromide (**9a**).* Light yellow solid, yield: 47%, m.p. 305–306 °C, IR cm^{-1}: 2622 (NH), 3193 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 7.27–7.58 (6H, m, Ar-H), 7.89 (2H, d, J = 8.4 Hz, Ar-H), 8.14–8.18 (1H, m, Ar-H), 8.41 (1H, d, J = 2.98 Hz, Ar-H), 8.83 (1H, s, Ar-H), 12.24 (1H, s, NH); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 106.0 (s), 110.9 (d), 112.6 (d), 120.3 (d), 121.6 (d), 123.3 (d), 123.7 (s), 124.6 (2xd), 127.7 (d), 128.8 (2xd), 130.0 (d), 132.1 (s), 136.7 (s), 142.6 (s), 142.8 (s), 158.2 (s). Anal. Calcd for $C_{18}H_{13}BrN_4S$ (MW 397.28): C, 54.42%; H, 3.30%; N, 14.10%. Found: C, 54.61%; H, 3.15%; N, 14.18%.

*5.1.4.2. 3-[6-(3-Methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole hydrobromide (**9b**).* Light yellow solid, yield: 43%, m.p. 284–285 °C, IR cm^{-1}: 2719 (NH), 3153 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.82 (3H, s, OCH_3), 6.87–6.92 (1H, m, Ar-H), 7.27–7.58 (6H, m, Ar-H), 8.14–8.18 (1H, m, Ar-H), 8.40 (1H, d, J = 4.6 Hz, Ar-H), 8.84 (1H, s, Ar-H), 10.44 (1H, s, NH), 12.23 (1H, s, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 55.1 (q), 106.0 (s), 109.9 (d), 111.1 (d), 112.6 (d), 113.3 (d), 116.9 (d), 120.3 (d), 121.6 (d), 123.3 (d), 123.7 (s), 129.9 (d), 130.0 (d), 133.6 (s), 136.7 (s), 142.5 (s), 142.7 (s), 158.2 (s), 159.6 (s). Anal. Calcd for $C_{19}H_{15}BrN_4OS$ (MW 427.33): C, 53.40%; H, 3.54%; N, 13.11%. Found: C, 53.51%; H, 3.50%; N, 13.23%.

*5.1.4.3. 3-[6-(2,5-Dimethoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole hydrobromide (**9c**).* Light yellow solid, yield: 42%, m.p. 260–261 °C, IR cm^{-1}: 2735 (NH), 3124 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.78 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 6.94–6.95 (1H, m, Ar-H), 7.09 (1H, d, J = 9.1 Hz, Ar-H), 7.28–7.32 (2H, m, Ar-H), 7.59–7.61 (2H, m, Ar-H), 8.15–8.20 (1H, m, Ar-H), 8.46 (1H, d, J = 3.15 Hz, Ar-H), 8.76 (2H, s, Ar-H, NH), 12.27 (1H, s, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 55.4 (q), 55.9 (q), 105.8 (s), 111.7 (d), 112.6 (d), 112.9 (d), 113.5 (d), 114.4 (d), 119.4 (s), 120.3 (d), 121.7 (d), 123.3 (d), 123.7 (s), 130.4 (d), 136.7 (s), 136.9 (s), 141.9 (s), 149.8 (s), 153.2 (s), 159.0 (s). Anal. Calcd for $C_{20}H_{17}BrN_4O_2S$ (MW 457.33): C, 52.52%; H, 3.75%; N, 12.25%. Found: C, 52.61%; H, 3.68%; N, 12.36%.

*5.1.4.4. 3-[6-(4-Fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole hydrobromide (**9d**).* Light yellow solid, yield: 42%, m.p. 318–319 °C, IR cm^{-1}: 2719 (NH), 3181 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 7.25–7.34 (4H, m, 4xAr-H), 7.53–7.57 (1H, m, Ar-H), 7.87–7.94 (2H, m, 2xAr-H), 8.12–8.16 (1H, m, Ar-H, NH), 8.40 (1H, d, J = 2.98 Hz, Ar-H), 8.79 (1H, s, Ar-H); 12.03 (1H, s, NH), 12.24 (1H, s, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 106.0 (s), 110.7 (d), 112.6 (d), 115.5 (d), 115.9 (d), 120.2 (d), 121.6 (d), 123.2 (d), 123.7 (s), 126.6 (d), 126.7 (d), 128.6 (s), 133.0 (d), 136.6 (s), 141.5 (s), 142.8 (s), 158.3 (s), 159.2 (s). Anal. Calcd for $C_{18}H_{12}BrFN_4S$ (MW 415.27): C, 52.06%; H, 2.91%; N, 13.49%. Found: C, 52.15%; H, 2.78%; N, 13.40%.

*5.1.4.5. 3-[6-(4-Nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole (**10e**).* Orange solid, yield: 52%, m.p. 359–360 °C, IR cm^{-1}: 3324 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 7.26–7.31 (2H, m, 2xAr-H), 7.51–7.56 (1H, m, Ar-H), 8.07–8.33 (6H, m, Ar-H), 8.93 (1H, s, Ar-H), 12.16 (1H, s, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 106.4 (s), 112.5 (d), 113.1 (d), 120.3 (d), 121.5 (d), 123.2 (d), 123.7 (s), 124.1 (2xd), 124.9 (2xd), 129.8 (d), 136.7 (s), 140.6 (s), 142.4 (s), 144.2 (s), 145.7 (s), 158.0 (s). Anal. Calcd for $C_{18}H_{11}N_5O_2S$ (MW 361.37): C, 59.82%; H, 3.07%; N, 19.38%. Found: C, 59.97%; H, 3.25%; N, 19.51%.

*5.1.4.6. 3-[6-(4-Trifluoromethylphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole (**10f**).* Light yellow solid, yield: 50%, m.p. 293–295 °C, IR cm^{-1}: 3678 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 7.27–7.32 (2H, m, 2xAr-H), 7.53–7.57 (1H, m, Ar-H), 7.77 (2H, d, J = 8.37, Ar-H), 8.08–8.20 (3H, m, Ar-H), 8.35 (1H, s, Ar-H), 8.88 (1H, s, Ar-H), 12.16 (1H, s, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 106.3 (s), 112.0 (s), 112.5 (d), 120.3 (s), 120.3 (d), 121.5 (d), 123.2 (d), 123.7 (d), 124.8 (2xd), 125.6 (2xd), 129.7 (d), 136.7 (s), 137.9 (s), 142.8 (s), 143.6 (s), 157.8 (s).

Anal. Calcd for $C_{19}H_{11}F_3N_4S$ (MW: 384.38): C, 59.37%; H, 2.88%; N, 14.58%. Found: C, 59.41%; H, 2.95%; N, 14.70%.

*5.1.4.7. 1-Methyl-3-(6-phenylimidazo[2,1-b][1,3,4]thiadiazol-2-yl)-1H-indole hydrobromide (**9g**).* Light yellow solid, yield: 50%, m.p. 282–283 °C, IR cm^{-1}: 2604 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.90 (3H, s, CH_3), 7.32–7.45 (5H, m, Ar-H), 7.60–7.64 (1H, m, Ar-H), 7.88 (2H, d, J = 4.22 Hz; Ar-H), 8.13–8.18 (1H, m, Ar-H), 8.40 (1H, s, Ar-H), 8.78 (1H, s, Ar-H), 9.14 (1H, bs, NH); ^{13}C (50 MHz, DMSO-d_6) δ: 33.1 (q), 100.0 (s), 110.8 (d), 111.1 (d), 120.4 (d), 121.9 (d),123.3 (d), 124.0 (s), 124.6 (2xd), 127.5 (d), 128.7 (2xd), 132.6 (s), 133.3 (d), 137.2 (s), 142.7 (s), 143.1 (s), 157.4 (s). Anal. Calcd for $C_{19}H_{15}BrN_4S$ (MW: 411.32): C, 55.48%; H, 3.68%; N, 13.62%. Found: C, 55.52%; H, 3.74%; N, 13.51%.

*5.1.4.8. 3-[6-(3-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1-methyl-1H-indole hydrobromide (**9h**).* Light yellow solid, yield: 52%, m.p. 266–267 °C, IR cm^{-1}: 2473 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.82 (3H, s, OCH_3), 3.90 (3H, s, CH_3), 6.86–6.91 (1H, m, Ar-H), 7.35–7.46 (5H, m, Ar-H, NH), 7.63–7.64 (1H, m, Ar-H), 8.13–8.17 (1H, m, Ar-H), 8.40 (1H, s, Ar-H), 8.80 (1H, s, Ar-H), 10.45 (1H, bs, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 33.1 (q), 55.0 (q), 105.0 (s), 109.9 (d), 111.0 (d), 112.0 (d), 113.1 (d), 116.9 (d), 120.4 (d), 121.9 (d), 123.2 (d), 124.0 (s),129.8 (d), 133.3 (d), 134.0 (s),

137.2 (s), 142.6 (s), 143.1 (s), 157.4 (s), 159.6 (s). Anal. Calcd for $C_{20}H_{17}BrN_4OS$ (MW: 441.34): C, 54.43%; H, 3.88%; N, 12.69%. Found: C, 54.51%; H, 3.99%; N, 12.82%.

5.1.4.9. 3-[6-(2,5-Dimethoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1-methyl-1H-indole hydrobromide ***(9i)***. Light yellow solid, yield: 48%, m.p. 266–267 °C, IR cm^{-1}: 2607 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.78 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.93 (3H, s, CH_3), 6.87–6.93 (1H, m, Ar-H), 7.02–7.10 (1H, m, Ar-H), 7.32–7.40 (2H, m, Ar-H), 7.61–7.64 (2H, m, Ar-H), 7.88 (1H, bs, NH), 8.14–8.18 (1H, m, Ar-H), 8.42 (1H, s, Ar-H), 8.67 (1H, s, Ar-H). ^{13}C NMR (50 MHz, DMSO-d_6) δ:33.1 (q), 55.4 (q), 55.9 (q), 105.7 (s), 111.7 (s), 112.6 (s), 112.9 (s), 113.5 (d), 114.3 (d), 119.4 (s), 120.2 (d), 121.7 (d), 123.3 (d), 123.6 (s), 130.4 (d), 136.7 (s), 136.9 (s), 141.9 (s), 149.8 (s), 153.2 (s), 159.0 (s). Anal. Calcd for $C_{21}H_{19}BrN_4O_2S$ (MW: 471.37): C, 53.51%; H, 4.06%; N, 11.89%. Found: C, 53.70%; H, 4.12%; N, 11.98%.

5.1.4.10. 3-[6-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1-methyl-1H-indole hydrobromide ***(9j)***. Light yellow solid, yield: 50%, m.p. 269–270 °C, IR cm^{-1}: 2610–2719 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.89 (3H, s, CH_3), 7.23–7.37 (4H, m, Ar-H), 7.59–7.63 (1H, m, Ar-H), 7.86–7.93 (2H, m, Ar-H), 8.12–8.21 (2H, m, Ar-H, NH), 8.37 (1H, s, Ar-H), 8.73 (1H, s, Ar-H); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 33.1 (q), 105.1 (s), 110.5 (d), 111.0 (d), 115.4 (d), 115.8 (d), 120.4 (d), 121.8 (d), 123.2 (d), 124.0 (s), 126.4 (d), 126.6 (d), 129.5 (s), 133.2 (d), 137.2 (s), 142.6 (2xs), 142.7 (s), 157.2 (s). Anal. Calcd for $C_{19}H_{14}BrFN_4S$ (MW: 429.31): C, 53.16%; H, 3.29%; N, 13.05%. Found: C, 53.08%; H, 3.88%; N, 12.72%.

5.1.4.11. 1-Methyl-3-[6-(4-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole ***(10k)***. Orange solid, yield: 52%, m.p. 299–300 °C, ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.92 (3H, s, CH_3), 7.34–7.38 (2H, m, 2xAr-H), 7.62–7.65 (1H, m, Ar-H), 8.12–8.40 (6H, m, Ar-H), 9.00 (1H, s, Ar-H). Anal. Calcd for $C_{19}H_{13}N_5O_2S$ (MW: 375.40): C, 60.79%; H, 3.49%; N, 18.66%. Found: C, 60.92%; H, 3.32%; N, 18.79%.

5.1.4.12. 1-Methyl-3-{6-[4-(trifluoromethyl)phenyl]imidazo[2,1-b][1,3,4]thiadiazol-2-yl}-1H-indole ***(10l)***. Light yellow solid, yield: 42% m.p. 242–243 °C, ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.88 (3H, s, CH_3), 7.30–7.39 (2H, m, Ar-H), 7.60 (1H, d, $J = 8.37$, Ar-H), 7.74 (2H, d, $J = 8.4$, Ar-H), 8.04–8.17 (3H, m, Ar-H), 8.32 (1H, s, Ar-H), 8.84 (1H, s, Ar-H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 33.1 (q), 105.2 (s), 111.0 (d), 112.0 (d), 112.1 (s), 120.4 (d), 120.6 (s), 121.8 (d), 123.2 (d), 124.0 (s), 124.8 (2xd), 125.5 (d), 125.6 (s), 133.2 (d), 137.2 (s), 137.7 (s), 142.8 (s), 143.4 (s), 157.3 (s). Anal. Calcd for $C_{20}H_{13}F_3N_4S$ (MW: 398.40): C, 60.29%; H, 3.29%; N, 14.06%. Found: C, 60.44%; H, 3.35%; N, 14.28%.

5.1.4.13. 5-Bromo-3-(6-phenylimidazo[2,1-b][1,3,4]thiadiazol-2-yl)-1H-indole hydrobromide ***(9m)***. Light yellow solid, yield: 50%, m.p. 303–304 °C, IR cm^{-1}: 2690–2804 (NH), 3553–3638 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 7.29–7.55 (5H, m, Ar-H), 7.87 (2H, d, $J = 7.6$ Hz, Ar-H), 8.37 (2H, d, $J = 18.81$ Hz, Ar-H), 8.81 (1H, s, Ar-H), 12.33 (1H, s, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 106.0 (s), 110.7 (d), 114.1 (s), 114.6 (d), 122.6 (d), 124.4 (2xd), 125.3 (s), 125.8 (d), 127.2 (d), 128.6 (2xd), 130.9 (d), 133.5 (s), 135.4 (s), 142.8 (s), 144.2 (s), 156.8 (s). Anal. Calcd for $C_{18}H_{12}Br_2N_4S$ (MW: 476.19): C, 45.40%; H, 2.54%; N, 11.77%. Found: C, 45.58%; H, 2.63%; N, 11.59%.

5.1.4.14. 5-Bromo-3-[6-(3-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole hydrobromide ***(9n)***. Light yellow solid, yield: 50%, m.p. 299–300 °C, IR cm^{-1}: 2629 (NH), 3113 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.81 (3H, s, OCH_3), 6.85–6.90 (1H, m, Ar-H), 7.34–7.54 (5H, m, Ar-H), 8.30 (1H, d, $J = 1.72$ Hz, Ar-H), 8.43 (1H, d, $J = 2.96$ Hz, Ar-H), 8.86 (1H, s, Ar-H), 10.16 (1H, bs, NH), 12.37 (1H, s, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 55.0 (q), 106.0 (s), 109.8 (d), 111.1 (d), 113.1 (d), 114.6 (d), 116.8 (d), 120.3 (s), 122.6 (d), 125.8 (d), 123.7 (s), 129.8 (d), 131.1 (d), 133.6 (s), 136.7 (s), 142.5 (s), 142.7 (s), 158.2 (s), 159.6 (s). Anal. Calcd for $C_{19}H_{14}Br_2N_4OS$ (MW: 506.21): C, 45.08%; H, 2.79%; N, 11.07%. Found: C, 45.16%; H, 2.88%; N, 11.15%.

5.1.4.15. 5-Bromo-3-[6-(2,5-dimethoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole hydrobromide ***(9o)***. Light yellow solid, yield: 43%, m.p. 306–307 °C, IR cm^{-1}: 2565–2645 (NH), 3604 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.77 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 6.89–6.91 (1H, m, Ar-H), 7.05 (1H, d, $J = 9$ Hz, Ar-H), 7.43–7.63 (3H, m, Ar-H), 8.32 (1H, d, $J = 1.7$ Hz, Ar-H), 8.46 (1H, d, $J = 2.98$ Hz, Ar-H), 8.72 (1H, s, Ar-H), 12.38 (1H, s, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 55.4 (q), 55.8 (q), 105.7 (s), 111.6 (d), 112.7 (d), 113.8 (d), 113.9 (s), 114.2 (d), 114.6 (s), 120.6 (s), 122.6 (d), 125.3 (d), 125.1 (d), 131.4 (d), 135.5 (s), 138.2 (s), 141.9 (s), 149.9 (s), 153.1 (s), 157.8 (s). Anal. Calcd for $C_{20}H_{16}Br_2N_4O_2S$ (MW: 536.24): C, 44.80%; H, 3.01%; N, 10.45%. Found: C, 44.71%; H, 3.20%, N, 10.56%.

5.1.4.16. 5-Bromo-3-[6-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole hydrobromide ***(9p)***. Light yellow solid, yield: 42%, m.p. 289–290 °C, IR cm^{-1}: 2868–2958 (NH), 3341–3484 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 7.22–7.54 (4H, m, Ar-H), 7.85–7.92 (2H, m, Ar-H), 8.36 (2H, d, $J = 26.23$ Hz, Ar-H), 8.78 (1H, s, Ar-H), 12.34 (1H, s, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 105.9 (s), 110.5 (d), 114.0 (s), 114.6 (d), 115.3 (d), 115.7 (d), 122.6 (d), 125.3 (s), 125.8 (d), 126.2 (d), 126.4 (d), 127.9 (s), 130.4 (s), 130.8 (d), 131.2 (s), 135.4 (s), 142.9 (s), 156.7 (s). Anal. Calcd for $C_{18}H_{11}Br_2FN_4S$ (MW: 494.18): C, 43.75%; H, 2.24%; N, 11.34%. Found: C, 43.82%; H, 2.18%; N, 11.51%.

5.1.4.17. 5-Bromo-3-[6-(4-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole ***(10q)***. Orange solid, yield: 42%, m.p. 337–338 °C, IR cm^{-1}: 3609 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 7.42–7.52 (2H, m, Ar-H), 8.10–8.34 (5H, m, Ar-H), 8.44 (1H, s, Ar-H), 9.09 (1H, s, Ar-H), 12.37 (1H, s, NH). Anal. Calcd for $C_{18}H_{10}BrN_5O_2S$ (MW: 440.27): C, 49.10%; H, 2.29%; N, 15.91%. Found: C, 49.25%; H, 2.03%; N, 15.79%.

5.1.4.18. 5-Bromo-3-[6-(4-trifluoromethylphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole ***(10r)***. White solid, yield: 45%, m.p. 314–315 °C, IR cm^{-1}: 3609 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 7.39–7.54 (2H, m, Ar-H), 7.76 (2H, d, $J = 8.27$ Hz, Ar-H), 8.02 (2H, d, $J = 8.03$ Hz, Ar-H), 8.35 (2H, dd, $J = 1.56$, 2.94 Hz, Ar-H), 8.94 (1H, s, Ar-H), 12.32 (1H, s, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 106.0 (s), 112.2 (d), 114.1 (s), 114.6 (s), 115.5 (d), 122.6 (d), 124.8 (2xd), 125.4 (s), 125.6 (s), 125.8 (d), 130.9 (d), 135.4 (s), 137.8 (s), 143.0 (s), 143.5 (s), 157.2 (s). Anal. Calcd for $C_{19}H_{10}BrF_3N_4S$ (MW: 463.27): C, 42.96%; H, 2.18%; N, 12.09%. Found: C, 43.12%; H, 2.35%; N, 12.28%.

5.1.4.19. 5-Bromo-1-methyl-3-(6-phenylimidazo[2,1-b][1,3,4]thiadiazol-2-yl)-1H-indole hydrobromide ***(9s)***. White solid, yield 52%, m.p. 219–220 °C, IR cm^{-1}: 2936 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.87 (3H, s, CH_3), 7.27–7.61 (5H, m, Ar-H), 7.84 (2H, d, $J = 7.19$ Hz, Ar-H), 8.26 (1H, d, $J = 1.81$ Hz, Ar-H), 8.40 (1H, d, $J = 5.2$ Hz, Ar-H), 8.81 (1H, s, Ar-H), 8.91 (1H, bs, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 33.4 (q), 104.7 (s), 110.9 (s) 113.2 (d), 114.6 (s), 122.6 (d), 124.5 (d), 125.5 (s), 125.8 (d), 127.5 (d), 128.7 (d), 132.8 (s), 134.5 (d), 136.0 (s), 142.6 (s), 143.4 (s), 156.8 (s). Anal. Calcd for $C_{19}H_{14}Br_2N_4S$ (MW: 490.21): C, 46.55%; H, 2.88%; N, 11.43%. Found: C, 46.78%; H, 2.95%; N, 11.51%.

5.1.4.20. 5-Bromo-3-[6-(3-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1-methyl-1H-indole hydrobromide ***(9t)***. Light yellow

solid, yield 45%, mp 285–286 °C, ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.80 (3H, s, CH_3), 3.86 (3H, s, OCH_3), 6.87 (1H, d, $J = 7.86$ Hz, Ar-H), 7.33–7.59 (5H, m, Ar-H), 8.24 (1H, d, $J = 1.78$ Hz, Ar-H), 8.41 (1H, s, Ar-H), 8.82 (1H, s, Ar-H), 9.50 (1H, bs, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 33.3 (q), 55.0 (q), 104.7 (s), 109.7 (d), 113.1 (d), 114.5 (s), 116.8 (d), 122.6 (d), 125.5 (s), 125.7 (d), 129.6 (d), 129.8 (d), 134.3 (d), 135.9 (s), 142.5 (s), 143.5 (s), 156.7 (s), 159.2 (s), 159.5 (s). Anal. Calcd for $C_{10}H_{16}Br_2N_4OS$ (MW: 520.24): C, 46.17%; H, 3.10%; N, 10.77%. Found: C, 46.28%; H, 3.02%; N, 10.91%.

5.1.4.21. 5-Bromo-3-[6-(2,5-dimethoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1-methyl-1H-indole hydrobromide ***(9u)****.* Light yellow solid, yield 53%, mp 290–291 °C, IR cm^{-1}: 2473–2610 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.77 (3H, s, CH_3), 3.88 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 6.39 (1H, bs, NH), 6.83–7.07 (2H, m, Ar-H), 7.44–7.63 (3H, m, Ar-H), 8.28 (1H, d, J = 1.68, Ar-H), 8.43 (1H, s, Ar-H), 8.66 (1H, s, Ar-H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 33.3 (q), 55.3 (q) 55.8 (q), 99.3 (s), 100.7 (d), 104.8 (s), 106.5 (d), 111.1 (s), 111.6 (d), 112.5 (d), 114.6 (s), 122.8 (d), 125.7 (d), 130.6 (d), 134.0 (d), 134.4 (s), 138.0 (s), 139.4 (s), 149.9 (s), 153.1 (s), 157.0 (s). Anal. Calcd for $C_{21}H_{18}Br_2N_4O_2S$ (MW: 550.26): C, 45.84%; H, 3.30%; N, 10.18%. Found: C, 45.93%; H, 3.60%; N, 10.32%.

5.1.4.22. 5-Bromo-3-[6-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1-methyl-1H-indole hydrobromide ***(9v)****.* White solid, yield 48%, mp 293–294 °C, IR cm^{-1}: 2588–2609 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.88 (3H, s, CH_3), 7.21–7.30 (2H, t, Ar-H), 7.55 (2H, m, Ar-H), 7.87 (2H, dd, $J = 1.9$, 8.69 Hz, Ar-H), 8.28 (1H, d, J = 1.62, Ar-H), 8.39 (1H, s, Ar-H), 8.75 (1H, s, Ar-H). Anal. Calcd for $C_{19}H_{13}Br_2FN_4S$ (MW: 508.20): C, 44.90%; H, 2.58%; N, 11.02%. Found: C, 45.06%; H, 2.64%; N, 11.13%.

5.1.4.23. 5-Bromo-3-[6-(4-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1-methyl-1H-indole hydrobromide ***(9w)****.* Orange solid, yield 46%, mp 320–321 °C, IR cm^{-1}: 2925 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.92 (3H, s, CH_3), 7.53 (1H, d, J = 2.08, Ar-H), 7.63–7.68 (1H, m, Ar-H), 8.12 (2H, d, J = 8.86, Ar-H), 8.28–8.33(3H, m, Ar-H), 8.47 (1H, s, Ar-H), 9.08 (1H, s, Ar-H). Anal. Calcd for $C_{19}H_{13}Br_2N_5O_2S$ (MW: 535.21): C, 42.64%; H, 2.45%; N, 13.08%. Found: C, 42.78%; H, 2.58%; N, 13.23%.

5.1.4.24. 5-Bromo-3-[6-(4-trifluoromethylphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1-methyl-1H-indole ***(10x)****.* Yellow solid, yield 42%, m.p. 254–255 °C, ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.84 (3H, s, CH_3), 7.41–7.57 (2H, m, 2xAr-H), 7.71 (2H, d, $J = 8.38$ Hz, Ar-H), 8.00 (2H, d, $J = 8.09$ Hz, Ar-H), 8.23 (1H, d, $J = 1.72$ Hz, Ar-H), 8.32 (1H, s, Ar-H), 8.85 (1H, s, Ar-H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 33.3 (q), 104.9 (s), 112.1 (d), 113.1 (d), 114.5 (s), 122.7 (2xd), 124.7 (d), 125.5 (d), 125.5 (d), 125.7 (d), 127.3 (s), 134.2 (d), 135.9 (s), 137.9 (s), 143.1 (s), 143.3 (s), 156.6 (s). Anal. Calcd for $C_{20}H_{12}BrF_3N_4S$ (MW: 477.30): C, 50.33%; H, 2.53%; N, 11.74%. Found: C, 50.54%; H, 2.72%; N, 11.88%.

5.1.4.25. 5-Chloro-3-(6-phenylimidazo[2,1-b][1,3,4]thiadiazol-2-yl)-1H-indole hydrobromide ***(9y)****.* Light yellow solid, yield: 45%, m.p. 297–298 °C, IR cm^{-1}: 2890 (NH), 3445 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 7.27–7.58 (5H, m, Ar-H), 7.87 (2H, d, $J = 7.14$ Hz, Ar-H), 8.17 (1H, d, $J = 1.82$ Hz, Ar-H), 8.40 (1H, d, $J = 2.86$ Hz, Ar-H), 8.76 (1H, s, Ar-H), 12.31 (1H, s, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 106.3 (s), 110.6 (d), 114.8 (d), 119.6 (d), 123.2 (d), 124.4 (2xd), 124.8 (s), 126.1 (s), 127.0 (d), 128.6 (2xd), 130.9 (d), 134.0 (s), 135.2 (s), 142.9 (s), 144.8 (s), 156.6 (s). Anal. Calcd for $C_{18}H_{12}BrClN_4S$ (MW 558.21): C, 50.08%; H, 2.80%; N, 12.98%. Found: C, 50.25%; H, 2.91%; N, 12.90%.

5.1.4.26. 5-Chloro-3-[6-(3-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole hydrobromide ***(9z)****.* White solid, yield: 46%, m.p. 291–292 °C, IR cm^{-1}: 3478 (NH), 2884 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.81 (3H, s, OCH_3), 6.88 (1H, d, $J = 6.84$ Hz, Ar-H), 7.28–7.59 (5H, m, Ar-H), 8.14 (1H, d, $J = 1.82$ Hz, Ar-H), 8.46 (1H, d, $J = 2.90$ Hz, Ar-H), 8.87 (1H, s, Ar-H), 10.19 (1H, bs, NH), 12.39 (1H, s, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 55.0 (q), 106.0 (s), 109.8 (d), 111.1 (s), 111.3 (s), 113.2 (d), 114.3 (2xd), 115.4 (s), 116.9 (d), 119.6 (s), 123.4 (d), 124.7 (s), 126.2 (s), 129.8 (d), 131.2 (s), 134.2 (s). Anal. Calcd for $C_{19}H_{14}BrClN_4OS$ (MW: 461.76): C, 49.42%; H, 3.06%; N, 12.13%. Found: C, 49.58%; H, 3.21%; N, 12.25%.

5.1.4.27. 5-Chloro-3-[6-(2,5-dimethoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole hydrobromide ***(9aa)****.* White solid, yield: 46%, mp 265–266 °C, IR cm^{-1}: 3473 (NH), 2902 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.77 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 6.86–6.92 (1H, m, Ar-H), 7.07 (1H, d, $J = 9.05$ Hz, Ar-H), 7.28–7.33 (1H, m, Ar-H), 7.57 (2H, m, Ar-H), 7.98 (1H, bs, NH), 8.15 (1H, s, Ar-H), 8.51 (1H, s, Ar-H), 8.76 (1H, s, Ar-H), 12.41 (1H, s, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 55.3 (q), 55.7 (q), 106.1 (s), 112.7 (d), 114.2 (s), 114.8 (d), 116.3 (s), 119.5 (d), 123.3 (d), 124.8 (s), 126.9 (s), 131.4 (d), 135.2 (s), 150.6 (s), 152.7 (s), 152.9 (d), 153.1 (s), 157.3 (s). Anal. Calcd for $C_{20}H_{16}BrClN_4O_2S$ (MW: 491.79): C, 48.84%; H, 3.28%; N, 11.39%. Found: C, 48.96%; H, 3.42%; N, 11.51%.

5.1.4.28. 5-Chloro-3-[6-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole hydrobromide ***(9 ab)****.* Light yellow solid, yield: 45%, m.p. 288–289 °C, IR cm^{-1}: 3484 (NH), 2890 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 7.20 (1H, s, NH), 7.23–7.33 (3H, m, Ar-H), 7.57 (1H, d, $J = 8.68$ Hz, Ar-H), 7.59 (1H, s, Ar-H), 7.86–7.90 (2H, m, Ar-H), 8.15 (1H, d, $J = 1.96$ Hz, Ar-H), 8.43 (1H, d, $J = 2.94$, Ar-H), 8.78 (1H, s, Ar-H), 12.34 (1H, s, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 106.1 (s), 110.6 (d), 114.2 (d), 115.4 (d), 115.8 (d), 119.6 (d), 123.3 (d), 124.7 (s), 126.1 (s), 126.3 (d), 126.5 (d), 130.0 (s), 131.1 (d), 135.2 (s),142.9 (s), 143.2 (s), 157.0 (s). Anal. Calcd for $C_{18}H_{11}BrClFN_4S$ (MW: 449.73): C, 48.07%, H, 2.47%, N, 12.46%. Found: C, 48.15%, H, 2.55%, N, 12.52%.

5.1.4.29. 5-Chloro-3-[6-(4-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole hydrobromide ***(9ac)****.* Yellow solid, yield: 42%, m.p. 342–343 °C, IR cm^{-1}: 3381 (NH), 2890 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 7.35 (1H, s, Ar-H), 7.58 (1H, d, $J = 8.84$ Hz, Ar-H), 8.15–8.47 (7H, m, Ar-H), 9.07 (1H, s, NH), 12.37 (1H, s, NH). Anal. Calcd for $C_{18}H_{11}BrClN_5O_2S$ (MW: 476.73): C, 45.35%, H, 2.33%, N, 14.69%. Found: C, 45.45%, H, 2.38%, N, 14.75%.

5.1.4.30. 5-Chloro-3-[6-(4-trifluoromethylphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole ***(10ad)****.* White solid, yield: 46%, m.p. 312–313 °C, IR cm^{-1}: 2884 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 7.28 (1H, d, $J = 6.96$ Hz, Ar-H), 7.55 (1H, d, $J = 6.96$ Hz, Ar-H), 7.74 (2H, d, $J = 8.12$ Hz, Ar-H), 8.02–8.14 (3H, m, Ar-H), 8.40 (1H, s, Ar-H), 8.92 (1H, s, Ar-H), 12.34 (1H, s, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 106.1 (s), 112.2 (d), 114.2 (d), 119.6 (d), 123.2 (d), 124.7 (s), 124.7 (s), 124.8 (2xd), 125.6 (d), 125.6 (d), 126.1 (s), 131.2 (d), 135.2 (s), 137.6 (s), 142.8 (s), 143.5 (s), 157.4 (s). Anal. Calcd for $C_{19}H_{10}ClF_3N_4S$ (MW: 418.82): C, 54.49%, H, 2.41%, N, 13.38%. Found: C, 54.56%, H, 2.52%, N, 13.43%.

5.1.4.31. 5-Chloro-1-methyl-3-(6-phenylimidazo[2,1-b][1,3,4]thiadiazol-2-yl)-1H-indole ***(10ae)****.* White solid, yield: 42%, m.p. 290–291 °C, ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.88 (3H, s, CH_3), 7.27–7.39 (4H, m, Ar-H), 7.43 (1H, d, $J = 7.18$ Hz, Ar-H), 7.64 (2H, d, $J = 8.76$ Hz, Ar-H), 8.13 (1H, s, Ar-H), 8.38 (1H, s, Ar-H), 8.72 (1H, s, Ar-H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 55.9 (q), 99.5 (s), 105.1 (s), 110.6 (d), 112.8 (d), 119.7 (d), 123.2 (d), 124.4 (d), 124.9 (d), 126.5 (s), 127.1 (2xd), 128.6 (d), 133.9 (s), 134.3 (d), 135.7 (s), 142.7 (s), 144.8 (s), 156.1(s). Anal. Calcd for $C_{19}H_{13}ClN_4S$ (MW: 364.85): C, 62.55%, H,

3.59%, N, 15.36%. Found: C, 62.76%, H, 3.81%, N, 15.42%.

*5.1.4.32. 5-Chloro-3-[6-(3-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1-methyl-1H-indole hydrobromide **(9af)**.* White solid, yield: 50%, m.p. 290–291 °C, IR cm^{-1}: 2970 (NH); ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.81 (3H, s, CH_3), 3.87 (3H, s, OCH_3), 6.87 (1H, d, $J = 6.78$ Hz, Ar-H), 7.33–7.41 (4H, m, Ar-H), 7.62 (1H, d, $J = 8.77$ Hz, Ar-H), 8.09 (1H, d, $J = 2.00$ Hz, Ar-H), 8.43 (1H, s, Ar-H), 8.81 (1H, s, Ar-H), 9.36 (1H, bs, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 33.4 (q), 55.0 (q), 104.8 (s), 109.8 (d), 111.1 (d), 112.8 (d), 113.2 (d), 116.9 (d), 119.6 (d), 123.2 (d), 124.9 (s), 126.6 (s), 129.8 (d), 134.0 (s), 134.6 (d), 135.7 (s), 142.5 (s), 143.2 (s), 156.9 (s), 159.6 (s). Anal. Calcd for $C_{20}H_{16}BrClN_4OS$ (MW: 475.79): C, 50.49%; H, 3.39%; N, 11.78%. Found: C, 50.62%; H, 3.48%; N, 11.90%.

*5.1.4.33. 5-Chloro-3-[6-(2,5-dimethoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1-methyl-1H-indole **(10ag)**.* White solid, yield: 55%, m.p. 289–290 °C. ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.76 (3H, s, CH_3), 3.91 (6H, s, 2xOCH_3), 6.80–6.86 (1H, m, Ar-H), 7.02 (1H, d, $J = 8.97$ Hz Ar-H), 7.36 (1H, dd, $J = 2.06$, 2.03 Hz, Ar-H), 7.63–7.72 (2H, m, Ar-H), 8.17 (1H, d, $J = 1.94$ Hz, Ar-H), 8.40 (1H, s, Ar-H), 8.52 (1H, s, Ar-H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 33.3 (q), 55.2 (q), 55.7 (q), 105.2 (s), 111.5 (d), 112.4 (d), 112.8 (d), 112.9 (d), 114.2 (d), 119.7 (d), 122.8 (s), 123.2 (d), 124.9 (s), 126.5 (s), 134.3 (d), 135.8 (s), 140.3 (s), 142.0 (s), 149.9 (s), 153.1 (s), 155.9 (s). Anal. Calcd for $C_{21}H_{17}ClN_4O_2S$ (MW: 424.90): C, 59.36%; H, 4.03%; N, 13.19%. Found: C, 59.52%; H, 4.28%; N, 13.30%.

*5.1.4.34. 5-Chloro-3-[6-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1-methyl-1H-indole **(10ah)**.* White solid, yield: 42%, m.p. 295–296 °C. ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.88 (3H, s, CH_3), 7.20–7.39 (3H, m, Ar-H), 7.64 (1H, d, $J = 8.76$ Hz, Ar-H), 7.84–7.91 (2H, m, Ar-H), 8.12 (1H, d, $J = 1.76$ Hz, Ar-H), 8.38 (1H, s, Ar-H), 8.70 (1H, s, Ar-H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 33.5 (q), 105.1 (s), 110.4 (d), 112.9 (d), 114.8 (s), 115.8 (d), 118.4 (d), 119.8 (d), 122.1 (d), 123.0 (d), 125.2 (s), 126.2 (d), 126.5 (s), 134.1 (d), 135.7 (s), 142.8 (s), 143.9 (s), 156.3 (s), 187.7 (s). Anal. Calcd for $C_{19}H_{12}ClFN_4S$ (MW: 382.84): C, 59.61%; H, 3.16%; N, 14.63%. Found: C, 59.82%; H, 3.24%; N, 14.90%.

*5.1.4.35. 5-Chloro-3-[6-(4-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1-methyl-1H-indole **(10ai)**.* Yellow solid, yield 48%, m.p. 315–316 °C. ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.91 (3H, s, CH_3), 7.38–7.43 (1H, m, Ar-H), 7.69 (1H, d, J = 8.77, Ar-H), 8.09–8.17 (3H, m, Ar-H), 8.29 (2H, d, J = 8.79, Ar-H), 8.48 (1H, s, Ar-H), 9.05 (1H, s, Ar-H). Anal. Calcd for $C_{19}H_{12}ClN_5O_2S$ (MW: 409.85): C, 59.61%; H, 3.16%; N, 14.63%. Found: C, 59.82%; H, 3.24%; N, 14.90%.

*5.1.4.36. 5-Chloro-3-[6-(4-trifluoromethylphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1-methyl-1H-indole **(10aj)**.* White solid, yield: 43%, m.p. 245–246 °C. ^{1}HNMR (200 MHz, DMSO-d_6) δ: 3.87 (3H, s, CH_3), 7.32–7.38 (1H, m, Ar-H), 7.61 (1H, d, $J = 8.77$, Ar-H) 7.73 (2H, d, $J = 8.34$ Hz, Ar-H), 8.01–8.11 (3H, m, Ar-H), 8.37 (1H, s, Ar-H), 8.86 (1H, s, Ar-H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 33.3 (q), 99.5 (s), 105.0 (s), 112.1 (d), 112.7 (d), 119.6 (d), 123.1 (d), 124.7 (2xd), 124.9 (s), 125.5 (2xd), 126.5 (s), 134.4 (d), 135.7 (s), 137.9 (s), 143.1 (s), 143.3 (s), 156.6 (s). Anal. Calcd for $C_{20}H_{12}ClF_3N_4S$ (MW: 432.85): C, 55.50%; H, 2.79%; N, 12.94%. Found: C, 55.71%; H, 2.90%; N, 12.88%.

5.2. Biology

5.2.1. Minimum inhibitory concentrations (MICs)

The following Gram-positive and Gram-negative bacterial reference strains were used: *S. aureus* ATCC 25923, *S. aureus* ATCC 6538 and *S. epidermidis* ATCC 12228, *P. aeruginosa* ATCC 15442 and *E. coli* ATCC 25922. MICs were determined by a previously described micromethod [10]. Briefly, a series of solutions were prepared with a range of concentrations from 100 to 0.75 μg/mL (obtained by two-fold serial dilution). The serial dilutions were made in tryptic soy broth (TSB, VWR International, Leuven) in a 96-wells plate, starting from a stock solution of 1 mg/mL in NaCl 0.9% w/v. A 10 μL volume of a bacterial suspension from a 24 h culture containing ~10^6 cfu/mL was added to each well. The plate was incubated at 37 °C for 24 h; after this time, the MICs were determined using a microplate reader (Glomax Multidetection System TM297 Promega, Milano, Italy) as the lowest concentrations of the studies compounds whose OD, read at 570 nm, was comparable with the negative control wells (broth only, without inoculum). Each assay was performed in triplicate and repeated at least twice.

5.2.2. Biofilm prevention assay

Above mentioned bacterial strains were incubated in test tubes with Tryptic Soy Broth (TSB) (5 mL) containing 2% w/v glucose at 37 °C for 24 h. The bacterial suspensions were then diluted to achieve a turbidity equivalent to a 0.5 McFarland standard. The diluted suspension (2.5 μL) was added to each well of a single cell culture polystyrene sterile, flat-bottom 96-well plate filled with TSB (100 μL) with 2% w/v glucose. A screening concentration of 100 μg/mL of all compounds were directly added to the wells to assess inhibition percentages values, or in the case of determination of BIC_{50} (the concentration at which the percentage of inhibition of biofilm formation is equal to 50% compared to the untreated control), concentrations in the range among 100 and 0.1 μg/mL. Plates were incubated at 37 °C for 24 h. After biofilm growth, the content of each well was removed, wells were washed twice with sterile PBS 1X and stained with 150 μL of 0.1% w/v crystal violet solution for 30 min at room temperature. Excess solution was removed and the plate was washed twice using tap water. A volume of 125 μL of acetic acid of 33% v/v was added for 15 min to each stained well to solubilize the dye.

The plate was read at 570 nm using a microplate reader (Glomax Multidetection System Promega). BIC_{50} were obtained by comparing the optical densities (ODs) of growth control wells with that of the sample wells, and the value was calculated by using a linear regression graph in Excel. Each assay was performed in triplicate and repeated at least twice.

The percentage of inhibition was calculated through the following formula:

$$\% \text{ of inhibition} = ((\text{OD growth control} - \text{OD sample})/\text{OD growth control}) \times 100$$

5.2.3. Anti-biofilm activity against preformed biofilm

A suspension of bacteria (0.5 McFarland standard) was obtained using the procedure described above for the inhibition of biofilm formation test. 2.5 μl of suspension was added to each well of a 96-weel plate containing TSB (100 μl) with 2% w/v glucose. After the growth of a biofilm (24 h old), the content of each well was removed, wells were washed up twice with sterile PBS and then filled with fresh TSB medium (200 μl). After that, different concentrations of compounds were added starting from a concentration equal or greater than MIC obtained against planktonic form of tested strains using TSB as medium. The microtiter plate was sealed and incubated at 37 °C for further 24 h. The content of each well was removed, wells were washed up twice with sterile PBS (100 μl to each well) and the 96-weel plate was placed at 60 °C for 1 h before staining with a 0.1% w/v crystal violet solution. After 30 min, plates were washed with tap water to remove any excess stain. Biofilm formation was determined by solubilizing crystal violet as

above described and the absorbance was read at 540 nm using a microplate reader (Glomax Multidetection System Promega). The percentages of inhibition were calculated with the above-reported. Each assay was performed in triplicate and repeated at least twice.

5.2.4. Screening as sortase A (SrtA) inhibitors

The compounds **9c**, **9h** and **9 ab**, showing the best activity in inhibiting biofilm formation of *S. aureus*, were tested at a screening concentration of 100 μM (1% DMSO) in black 96-well plates (Greiner Bio-One) as SrtA inhibitors. A known SrtA inhibitor, 4-(hydroxymercuri)benzoic acid, was used as positive control. The inhibitory activity of the three compounds was evaluated by quantifying the increase in fluorescence intensity upon cleavage of the Fluorescence Resonance Energy Transfer (FRET) peptide substrate into two separate fragments resulting in the release of 5-Fam fluorescence, which can be monitored at excitation/emission = 490/520 nm. A commercial kit (SensoLyte® 520 Sortase A Activity Assay Kit * Fluorimetric*) was used with slight modifications. Briefly, the reactions were performed in a volume of 100 μl containing 1X assay buffer, 2.5 μg/ml SrtA protease recombinant, 4 μM fluorescent peptide substrate, and the prescribed concentrations of the test compounds or positive control. The peptide substrate without the recombinant SrtA was incubated under the same conditions, and used as a negative control. The reactions were conducted adding both the test compounds and the diluted enzyme solution to the microplate wells. Then sortase substrate solution was added into each well. For kinetic reading, immediately start measuring fluorescence at Ex/Em = 490/520 nm continuously recording data every 5 min for 60 min. All fluorescence-reading results are expressed in relative fluorescence units (RFU).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2019.02.007.

References

[1] S. Cascioferro, The future of antibiotic: from the magic bullet to the smart bullet, J. Microb. Biochem. Technol. 06 (2014), https://doi.org/10.4172/1948-5948.1000e118.

[2] S. Cascioferro, M.G. Cusimano, D. Schillaci, Antiadhesion agents against Gram-positive pathogens, Future Microbiol. 9 (2014) 1209–1220, https://doi.org/10.2217/fmb.14.56.

[3] D. Schillaci, V. Spanò, B. Parrino, A. Carbone, A. Montalbano, P. Barraja, P. Diana, G. Cirrincione, S. Cascioferro, Pharmaceutical approaches to target antibiotic resistance mechanisms, J. Med. Chem. 60 (2017) 8268–8297, https://doi.org/10.1021/acs.jmedchem.7b00215.

[4] M. Jamal, W. Ahmad, S. Andleeb, F. Jalil, M. Imran, M.A. Nawaz, T. Hussain, M. Ali, M. Rafiq, M.A. Kamil, Bacterial biofilm and associated infections, J. Chin. Med. Assoc. JCMA. 81 (2018) 7–11, https://doi.org/10.1016/j.jcma.2017.07.012.

[5] A.S. Lynch, G.T. Robertson, Bacterial and fungal biofilm infections, Annu. Rev. Med. 59 (2008) 415–428, https://doi.org/10.1146/annurev.med.59.110106.132000.

[6] B. Parrino, P. Diana, G. Cirrincione, S. Cascioferro, Bacterial biofilm inhibition in the development of effective anti-virulence strategy, Open Med. Chem. J. 12 (2018), https://doi.org/10.2174/1874104501812010084.

[7] M.V. Raimondi, B. Maggio, D. Raffa, F. Plescia, S. Cascioferro, G. Cancemi, D. Schillaci, M.G. Cusimano, M. Vitale, G. Daidone, Synthesis and anti-staphylococcal activity of new 4-diazopyrazole derivatives, Eur. J. Med. Chem. 58 (2012) 64–71, https://doi.org/10.1016/j.ejmech.2012.09.041.

[8] D. Schillaci, S. Petruso, M.V. Raimondi, M.G. Cusimano, S. Cascioferro, M. Scalisi, M.A. La Giglia, M. Vitale, Pyrrolomycins as potential anti-staphylococcal biofilms agents, Biofouling 26 (2010) 433–438, https://doi.org/10.1080/08927011003718673.

[9] D. Schillaci, S. Petruso, S. Cascioferro, M.V. Raimondi, J. a. J. Haagensen, S. Molin, In vitro anti-Gram-positive and antistaphylococcal biofilm activity of newly halogenated pyrroles related to pyrrolomycins, Int. J. Antimicrob. Agents 31 (2008) 380–382, https://doi.org/10.1016/j.ijantimicag.2007.10.013.

[10] D. Schillaci, A. Spinello, M.G. Cusimano, S. Cascioferro, G. Barone, M. Vitale, V. Arizza, A peptide from human β thymosin as a platform for the development of new anti-biofilm agents for Staphylococcus spp. and Pseudomonas aeruginosa, World J. Microbiol. Biotechnol. 32 (2016) 124, https://doi.org/10.1007/s11274-016-2096-2.

[11] J.S. Park, E.-J. Ryu, L. Li, B.-K. Choi, B.M. Kim, New bicyclic brominated furanones as potent autoinducer-2 quorum-sensing inhibitors against bacterial biofilm formation, Eur. J. Med. Chem. 137 (2017) 76–87, https://doi.org/10.1016/j.ejmech.2017.05.037.

[12] K. Sambanthamoorthy, R.E. Sloup, V. Parashar, J.M. Smith, E.E. Kim, M.F. Semmelhack, M.B. Neiditch, C.M. Waters, Identification of small molecules that antagonize diguanylate cyclase enzymes to inhibit biofilm formation, Antimicrob. Agents Chemother. 56 (2012) 5202–5211, https://doi.org/10.1128/AAC.01396-12.

[13] B. Parrino, D. Schillaci, I. Carnevale, E. Giovannetti, P. Diana, G. Cirrincione, S. Cascioferro, Synthetic small molecules as anti-biofilm agents in the struggle against antibiotic resistance, Eur. J. Med. Chem. 161 (2019) 154–178, https://doi.org/10.1016/j.ejmech.2018.10.036.

[14] M.A.A. Radwan, E.A. Ragab, N.M. Sabry, S.M. El-Shenawy, Synthesis and biological evaluation of new 3-substituted indole derivatives as potential anti-inflammatory and analgesic agents, Bioorg. Med. Chem. 15 (2007) 3832–3841, https://doi.org/10.1016/j.bmc.2007.03.024.

[15] M. Giampieri, A. Balbi, M. Mazzei, P. La Colla, C. Ibba, R. Loddo, Antiviral activity of indole derivatives, Antivir. Res. 83 (2009) 179–185, https://doi.org/10.1016/j.antiviral.2009.05.001.

[16] B. Parrino, A. Carbone, V. Spanò, A. Montalbano, D. Giallombardo, P. Barraja, A. Attanzio, L. Tesoriere, C. Sissi, M. Palumbo, G. Cirrincione, P. Diana, Azaisoindolo and isoindolo-azaquinoxaline derivatives with antiproliferative activity, Eur. J. Med. Chem. 94 (2015) 367–377, https://doi.org/10.1016/j.ejmech.2015.03.009.

[17] V. Spanò, A. Attanzio, S. Cascioferro, A. Carbone, A. Montalbano, P. Barraja, L. Tesoriere, G. Cirrincione, P. Diana, B. Parrino, Synthesis and antitumor activity of new thiazole nortopsentin analogs, Mar. Drugs 14 (12) (2016) 14120226, https://doi.org/10.3390/md14120226.

[18] B. Parrino, A. Attanzio, V. Spanò, S. Cascioferro, A. Montalbano, P. Barraja, L. Tesoriere, P. Diana, G. Cirrincione, A. Carbone, Synthesis, antitumor activity and CDK1 inhibiton of new thiazole nortopsentin analogues, Eur. J. Med. Chem. 138 (2017) 371–383, https://doi.org/10.1016/j.ejmech.2017.06.052.

[19] B. Parrino, A. Carbone, C. Ciancimino, V. Spanò, A. Montalbano, P. Barraja, G. Cirrincione, P. Diana, C. Sissi, M. Palumbo, O. Pinato, M. Pennati, G. Beretta, M. Folini, P. Matyus, B. Balogh, N. Zaffaroni, Water-soluble isoindolo[2,1-a]quinoxalin-6-imines: in vitro antiproliferative activity and molecular mechanism(s) of action, Eur. J. Med. Chem. 94 (2015) 149–162, https://doi.org/10.1016/j.ejmech.2015.03.005.

[20] V. Sharma, P. Kumar, D. Pathak, Biological importance of the indole nucleus in recent years: a comprehensive review, J. Heterocycl. Chem. 47 (2010) 491–502, https://doi.org/10.1002/jhet.349.

[21] J.-H. Lee, J. Lee, Indole as an intercellular signal in microbial communities, FEMS Microbiol. Rev. 34 (2010) 426–444, https://doi.org/10.1111/j.1574-6976.2009.00204.x.

[22] J. Lee, C. Attila, S.L.G. Cirillo, J.D. Cirillo, T.K. Wood, Indole and 7-hydroxyindole diminish Pseudomonas aeruginosa virulence, Microb. Biotechnol. 2 (2009) 75–90, https://doi.org/10.1111/j.1751-7915.2008.00061.x.

[23] H.H. Lee, M.N. Molla, C.R. Cantor, J.J. Collins, Bacterial charity work leads to population-wide resistance, Nature 467 (2010) 82–85, https://doi.org/10.1038/nature09354.

[24] J.-H. Lee, M.H. Cho, J. Lee, 3-indolylacetonitrile decreases Escherichia coli O157:H7 biofilm formation and Pseudomonas aeruginosa virulence, Environ. Microbiol. 13 (2011) 62–73, https://doi.org/10.1111/j.1462-2920.2010.02308.x.

[25] R. Romagnoli, P.G. Baraldi, F. Prencipe, J. Balzarini, S. Liekens, F. Estévez, Design, synthesis and antiproliferative activity of novel heterobivalent hybrids based on imidazo[2,1-b][1,3,4]thiadiazole and imidazo[2,1-b][1,3]thiazole scaffolds, Eur. J. Med. Chem. 101 (2015) 205–217, https://doi.org/10.1016/j.ejmech.2015.06.042.

[26] K. Jakovljević, I.Z. Matić, T. Stanojković, A. Krivokuća, V. Marković, M.D. Joksović, N. Mihailović, M. Nićiforović, L. Joksović, Synthesis, antioxidant and antiproliferative activities of 1,3,4-thiadiazoles derived from phenolic acids, Bioorg. Med. Chem. Lett 27 (2017) 3709–3715, https://doi.org/10.1016/j.bmcl.2017.07.003.

[27] S.G. Alegaon, K.R. Alagawadi, P.V. Sonkusare, S.M. Chaudhary, D.H. Dadwe, A.S. Shah, Novel imidazo[2,1-b][1,3,4]thiadiazole carrying rhodanine-3-acetic acid as potential antitubercular agents, Bioorg. Med. Chem. Lett 22 (2012) 1917–1921, https://doi.org/10.1016/j.bmcl.2012.01.052.

[28] B.A. Bhongade, S. Talath, R.A. Gadad, A.K. Gadad, Biological activities of imidazo[2,1-b][1,3,4]thiadiazole derivatives: a review, J. Saudi Chem. Soc. 20 (2016) S463–S475, https://doi.org/10.1016/j.jscs.2013.01.010.

[29] V.B. Jadhav, M.V. Kulkarni, V.P. Rasal, S.S. Biradar, M.D. Vinay, Synthesis and anti-inflammatory evaluation of methylene bridged benzofuranyl imidazo[2,1-b][1,3,4]thiadiazoles, Eur. J. Med. Chem. 43 (2008) 1721–1729, https://doi.org/10.1016/j.ejmech.2007.06.023.

[30] B. Chandrakantha, A.M. Isloor, P. Shetty, H.K. Fun, G. Hegde, Synthesis and biological evaluation of novel substituted 1,3,4-thiadiazole and 2,6-di aryl substituted imidazo [2,1-b] [1,3,4] thiadiazole derivatives, Eur. J. Med. Chem. 71 (2014) 316–323, https://doi.org/10.1016/j.ejmech.2013.10.056.

[31] H. Tahtaci, H. Karacık, A. Ece, M. Er, M.G. Şeker, Design, synthesis, SAR and molecular modeling studies of novel imidazo[2,1-b][1,3,4]thiadiazole derivatives as highly potent antimicrobial agents, Mol. Inform. 37 (2018),

https://doi.org/10.1002/minf.201700083.
[32] J. Zhang, H. Liu, K. Zhu, S. Gong, S. Dramsi, Y.-T. Wang, J. Li, F. Chen, R. Zhang, L. Zhou, L. Lan, H. Jiang, O. Schneewind, C. Luo, C.-G. Yang, Antiinfective therapy with a small molecule inhibitor of Staphylococcus aureus sortase, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 13517–13522, https://doi.org/10.1073/pnas.1408601111.
[33] S. Cascioferro, M. Totsika, D. Schillaci, A. Sortase, An ideal target for anti-virulence drug development, Microb. Pathog. 77C (2014) 105–112, https://doi.org/10.1016/j.micpath.2014.10.007.
[34] S. Cascioferro, D. Raffa, B. Maggio, M.V. Raimondi, D. Schillaci, G. Daidone, Sortase a inhibitors: recent advances and future perspectives, J. Med. Chem. 58 (2015) 9108–9123, https://doi.org/10.1021/acs.jmedchem.5b00779.
[35] A. Carbone, B. Parrino, M.G. Cusimano, V. Spanò, A. Montalbano, P. Barraja, D. Schillaci, G. Cirrincione, P. Diana, S. Cascioferro, New thiazole nortopsentin analogues inhibit bacterial biofilm formation, Mar. Drugs 16 (2018), https://doi.org/10.3390/md16080274.
[36] S. Cascioferro, B. Maggio, D. Raffa, M.V. Raimondi, M.G. Cusimano, D. Schillaci, B. Manachini, A. Leonchiks, G. Daidone, A new class of phenylhydrazinylidene derivatives as inhibitors of Staphylococcus aureus biofilm formation, Med. Chem. Res. 25 (2016) 870–878, https://doi.org/10.1007/s00044-016-1535-9.
[37] S. Cascioferro, B. Maggio, D. Raffa, M.V. Raimondi, M.G. Cusimano, D. Schillaci, B. Manachini, F. Plescia, G. Daidone, Synthesis and biofilm formation reduction of pyrazole-4-carboxamide derivatives in some Staphylococcus aureus strains, Eur. J. Med. Chem. 123 (2016) 58–68, https://doi.org/10.1016/j.ejmech.2016.07.030.
[38] A. Carbone, B. Parrino, G. Di Vita, A. Attanzio, V. Spanò, A. Montalbano, P. Barraja, L. Tesoriere, M.A. Livrea, P. Diana, G. Cirrincione, Synthesis and antiproliferative activity of thiazolyl-bis-pyrrolo[2,3-b]pyridines and indolyl-thiazolyl-pyrrolo[2,3-c]pyridines, nortopsentin analogues, Mar. Drugs 13 (2015) 460–492, https://doi.org/10.3390/md13010460.
[39] B. Parrino, A. Carbone, G. Di Vita, C. Ciancimino, A. Attanzio, V. Spanò, A. Montalbano, P. Barraja, L. Tesoriere, M.A. Livrea, P. Diana, G. Cirrincione, 3-[4-(1H-indol-3-yl)-1,3-thiazol-2-yl]-1H-pyrrolo[2,3-b]pyridines, nortopsentin analogues with antiproliferative activity, Mar. Drugs 13 (2015) 1901–1924, https://doi.org/10.3390/md13041901.
[40] B. Parrino, C. Ciancimino, A. Carbone, V. Spano, A. Montalbano, P. Barraja, G. Cirrincione, P. Diana, Synthesis of Isoindolo[1,4]benzoxazinone and Isoindolo[1,5]benzoxazepine: Two New Ring Systems of Pharmaceutical Interest, vol. 71, 2015, pp. 7332–7338, https://doi.org/10.1016/j.tet.2015.04.083.
[41] B. Parrino, S. Ullo, A. Attanzio, V. Spanò, S. Cascioferro, A. Montalbano, P. Barraja, L. Tesoriere, G. Cirrincione, P. Diana, New tripentone analogs with antiproliferative activity, Mol. Basel Switz. 22 (2017), https://doi.org/10.3390/molecules22112005.
[42] R.J. Melander, M.J. Minvielle, C. Melander, Controlling bacterial behavior with indole-containing natural products and derivatives, Tetrahedron 70 (2014) 6363–6372, https://doi.org/10.1016/j.tet.2014.05.089.
[43] N.D. Adams, C.J. Aquino, A.M. Chaudhari, J.M. Ghergurovich, T.J. Kiesow, C.A. Parrish, A.J. Reif, K. Wiggall, Triazolones as Fatty Acid Synthase Inhibitors, EP2538787A1, 2013.
[44] Xinglong Jiang, Ashish Tiwari, Maethonia Thompson, Zhihong Chen, Thomas P. Cleary, T.B.K. Lee, A practical method for N-methylation of indoles using dimethyl carbonate, Org. Proc. Res. Dev. 5 (6) (2001) 604–608, https://doi.org/10.1021/op0102215.