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Original article

Thiazolidione derivatives as novel antibiofilm agents: Design, synthesis, biological evaluation, and structure–activity relationships

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ABSTRACT

Rational designed novel thiazolidiones were synthesized and evaluated for antibiofilm activity. The active derivatives were not only potent inhibitors of *Staphylococcus epidermidis* biofilm growth but also efficient antibacterial agents. **3f** showed 4-fold higher activity (6.25 μM) in the biofilms dispersal assay and significantly higher antibacterial activity (MIC 3.125 μM) in comparison to the 3-(5-((6- (ethoxycarbonyl)-5-(benzo[1,3]dioxol-5-yl)-3-oxo-7-phenyl- thiazolo[3,2-a]pyrimidin-2(5H)-ylidene)methyl) furan-2-yl)benzoic acid (**1**).

1. Introduction

Bacterial biofilms are known to infect patients with in-dwelling medical devices (IMDs) such as cardiac pacemakers and heart stents [1–3]. They also possess increased resistance to host immune responses and antibiotics, often representing a significant challenge to overcome by the increased morbidity and mortality rates of numerous biofilm-mediated diseases [4–7]. Recent estimates have attributed biofilm-associated infections as being responsible for upward of 75% of microbial infections. Given the breadth of biofilm-mediated infections, there is a significant need for novel antibiofilm modulators.

As shown in Fig. 1, compound **1** derived from the thiazolidione class is a pioneering antibiofilm agent which is not only a moderate inhibitor of *Staphylococcus epidermidis* (ATCC35984) biofilm growth but also efficient antibacterial agent [8]. It elicits effect through inhibition of the YycG histidine kinase, which is an important control protein of two-component system (TCS) YycG/YycF regulated biofilm formation positively. Its unique mechanism of action underlies the efficacy and low risk of side effects. However, the improvement of antibiofilm potency of **1** is still needed and its structure modification is a challenging task.

We have recently focused on the development of straightforward chemistry to access analogues of thiazolidione for antibiofilm screening [9]. In a continued effort, we sought to a rational design to modify functional groups and to alter scaffold of compound **1** in generating derivatives to improve the antibiofilm and antibacterial activities. Additionally, the structure–activity relationships (SAR) of the thiazolidione family in the context of antibiofilm activity were also critical problems which we attempted to probe in this article.

In a first effort to optimize the activity of compound **1** (Scheme 1), C-5 position was evaluated utilizing phenyl and 4-methoxy phenyl substituents (**1a**–**b**). For C-2 position methyl 3-(5-formylfuran-2-yl)benzoate fragment (**1c**–**d**) and diverse substituted phenyl groups were introduced (**1e**–**j**). To further explore features which might be necessary for antibiofilm activity of compound **1**, we paid attention to phenoxy acetic acid derivatives (PAADs). PAADs were reported as potent antimycobacterial agents [10]. Following the same rationale of multitarget-directed ligands

Abbreviations: IMDs, in-dwelling medical devices; TCS, two-component system; PAADs, phenoxy acetic acid derivatives; SAR, structure–activity relationship; MTDLs, multitarget-directed ligands; DHPMs, dihydropyrimidine scaffold; PBS, phosphate buffered saline; TSB, tryptic soy broth; MIC, minimal inhibitory concentration; CLSI, Clinical and Laboratory Standards Institute.

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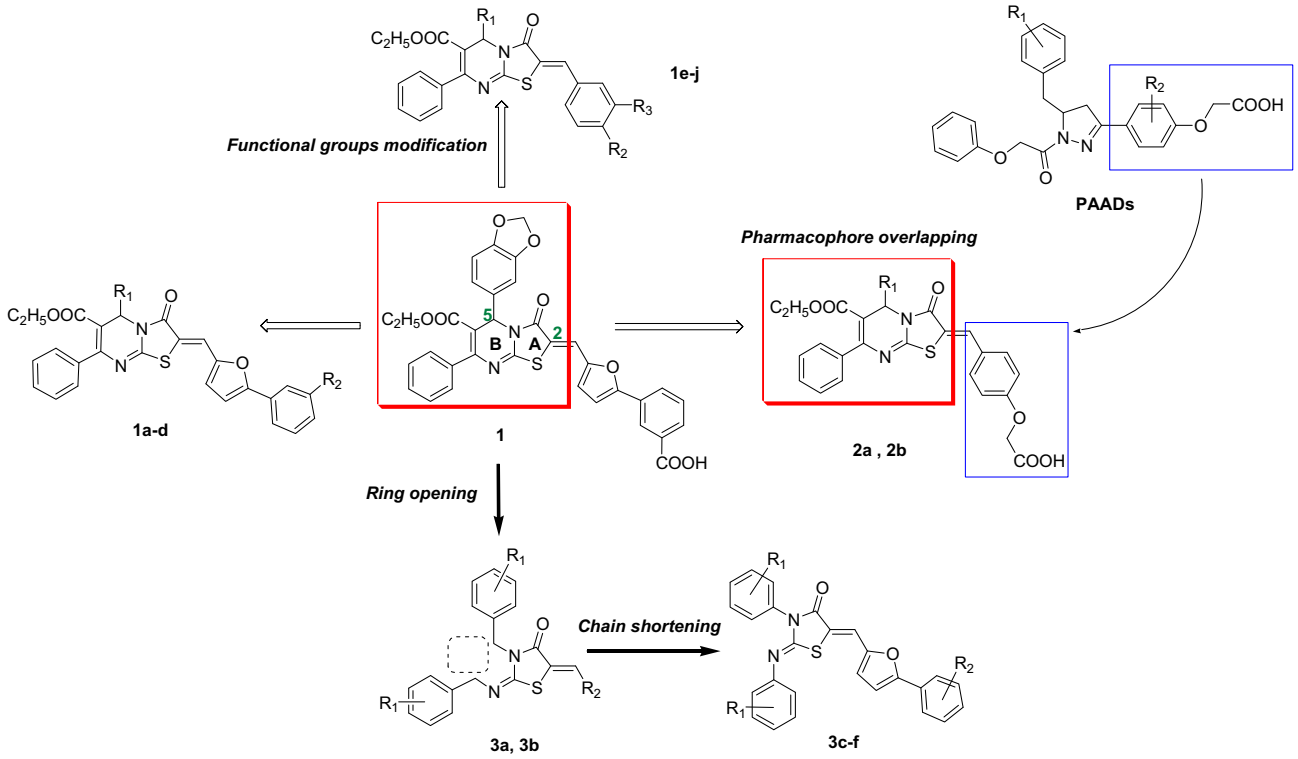
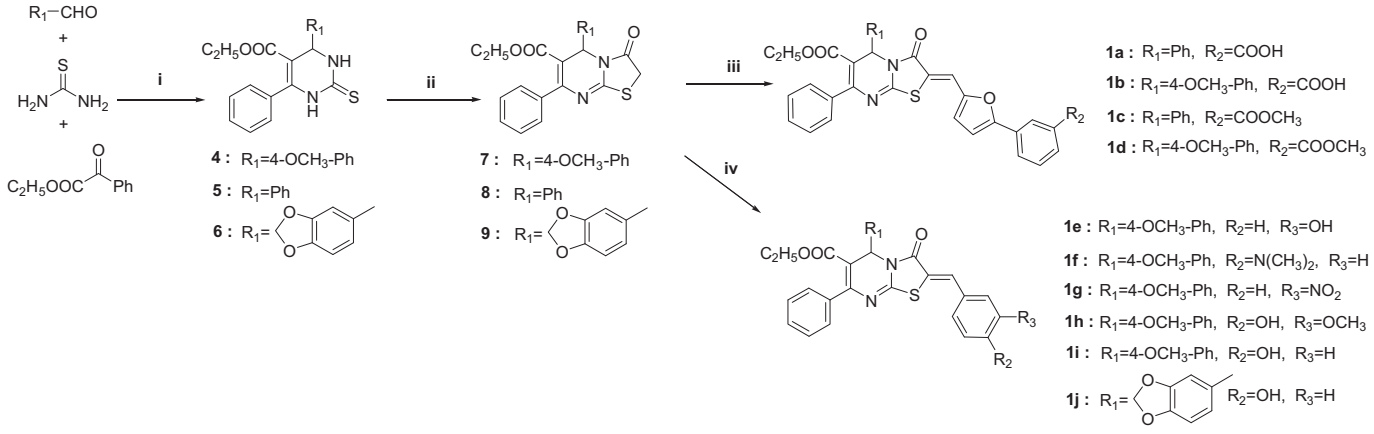


Fig. 1. Design Strategy.

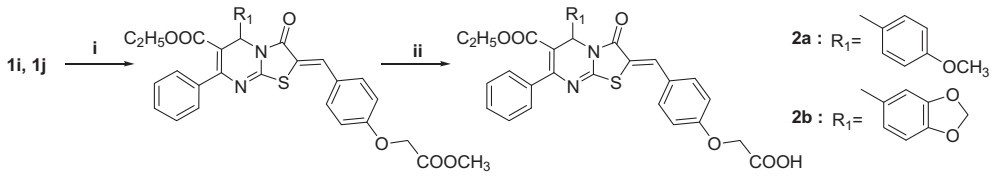
(MTDLs) [11], we envisioned that merging the antimycobacterial features of PAADs and antibiofilm features of compound **1** in a single chemical entity could, hopefully, lead to agents with multiple antibacterial mechanisms, hence a better potential for prevention and therapy of biofilm-mediated diseases. Therefore, we replaced one terminal 3-(5-formylfuran-2-yl)benzoic acid function group of **1** with a structure-similar 2-phenoxyacetic acid fragment to afford new compounds (**2a**, **2b**). The structure of PAADs also inspired that opening the B-ring of compound **1** but keep its thiazolidione core structure stability would furnish a novel class of compounds contained more flexible aryl side chains connecting with N atoms (**3a**–**b**). To further explore the SAR, derivatives **3c**–**f** were designed and synthesized by shortening the chain length between N atoms and phenyl groups.

2. Chemistry

The general synthesis of **1a**–**j** was shown in Scheme 1. A simple one-pot reaction known as Biginelli reaction of phenylaldehyde or substituted phenylaldehyde, ethyl benzoylacetate and thiourea gave different dihydropyrimidine scaffolds (DHPMs, **4** ,**5** and **6**) [12]. Cyclization of the three DHPMs with chloracetic acid afforded **7**, **8** and **9** [13]. An aldol condensation of substituted aromatic aldehydes with equivalent amount of **7** (**8** or **9**) was catalysed by acid or base to give **1a**–**j** in good yields [14,15]. As shown in Scheme 2, compounds **2a** and **2b** were readily prepared in two steps from **1i** and **1j** [16–18]. Cyclization reaction, aldol condensation, substitution reaction and hydrolization were efficiently used to synthesize **3a**–**f**, as shown in Scheme 3 [9,15].



Scheme 1. Reagents and conditions: (i) $SnCl_2 \cdot 2H_2O$, EtOH, reflux; (ii) chloroacetic acid, NaOAc, HAc/Ac_2O, reflux; (iii) β-alanine, acetic acid, reflux; (iv) piperidine, absolute alcohol, 60 °C.



Scheme 2. Reagents and conditions: (i) $BrCH_2COOCH_3$, acetone, reflux; (ii) K_2CO_3, water, methanol, reflux.

3. Results and discussion

Among tested compounds (Table 1), **1a**–**j** did not yield any major improvements. Only C-5 position 4-methoxy phenyl derivative **1b** displayed comparable antibiofilm and antibacterial activities to compound **1**. Nitrophenyl, hydroxyphenyl, dimethylaminophenyl and methyl 3-(5-formylfuran-2-yl) benzoate fragment introduced into C-2 position led bioactivity disappeared (>200 μM). By contrast, **2a** and **2b** were both less potent agents than compound **1**, with antibiofilm concentrations of 50 μM and MIC values of 25 μM, respectively. These data indicated that 3-(5-formylfuran-2-yl)benzoic acid fragment might be a more potent moiety than 2-phenoxyacetic acid fragment. The rigidity of the large moiety, probably, was one of the factors led the enhancement of activity.

Interestingly, the ring-opening derivative **3a** was a more potent antibiofilm and antibacterial agent than **2a**, with antibiofilm concentration 25 μM and MIC 12.5 μM, respectively. Comparing with **1b**, the corresponding ring-opening compound **3b** was a less potent agent. The activity data of **3a** and **3b** showed that B-ring opening analogs of *thiazolo[3,2-a]pyrimidin* **1** maintained the antibiofilm and antibacterial potency. Surprisingly, the chain-shortening analogs **3c**–**f** (derivatives of **3b**) showed excellent antibiofilm activities among the tested compounds. In comparison with compound **3b**, the antibiofilm concentration and MIC of **3c** were improved in 8-fold (showing 6.25 μM and 6.25 μM, respectively), and this improvement cued that the activity increased when substituted phenyl moieties linked with N atoms directly. The most potent inhibitor **3f** showed a 4-fold higher activity (6.25 μM) in the ATCC35984 biofilms dispersal assay and significantly higher antibacterial activity (MIC = 3.125 μM) in comparison to compound **1**. It was also notable that replacing the 3-benzoic acid substituent (**3e**) with 4-benzoic acid substituent (**3f**) resulted in a 4-fold improvement in its antibiofilm concentration (6.25 μM) and MIC (3.125 μM). All the results showed that fragments connected with C-2 position and the substituent phenyl moieties connected with N atoms might be two decisive factors influenced the activity.

4. Conclusion

A series of thiazolidione derivatives had been designed, synthesized and investigated as antibiofilm agents to the *S. epidermidis*, several compounds exceeded or maintained the potency of known biofilms inhibitor *thiazolo[3,2-a]pyrimidin* **1**. In this context, some points were noteworthy: (1) B-ring opening analogs of *thiazolo[3,2-a]pyrimidin* **1** maintained the antibiofilm potency, (2) substituent phenyl moieties which connected with N atoms obviously influenced antibiofilm activity, (3) the large-sized carboxylic acid moieties such as 4-(5-formylfuran-2-yl)benzoic acid fragment which replaced nitrophenyl, hydroxyphenyl or dimethylaminophenyl at C-2 position obtained excellent activity. Furthermore, the most promising results were observed for compound **3f**, with potent antibiofilm activity (6.25 μM) and high antibacterial activity (MIC = 3.125 μM). In compare with **1**, the chemical structure of **3f** was simpler and its activity was improved in 4-fold. These findings provide important pharmacophore information for the exploration of drug-like antibiofilm agents and further studies are on going in our laboratories.

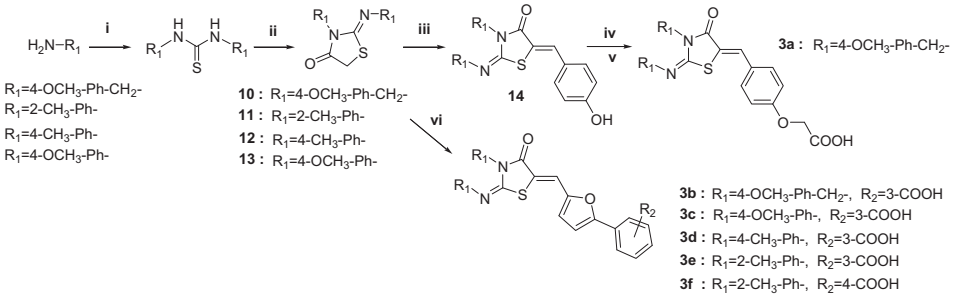
5. Experimental

All reagents and solvents were commercially available and used without further purification. Melting points were determined on an electrothermal digital apparatus model WRS-1B (Shanghai, China), without correction. Infrared spectra were recorded on an Avatar 360 ESP spectrometer (Nicolet), using potassium bromide (1% w/w) disk scanning from 500 to 4000 cm^{-1}. A Bruker AM-500 MHz instrument (Bruker) was used to acquire ^1H NMR and ^{13}C NMR spectra with TMS as internal standard. Chloroform-D and DMSO-d6 were used as solvents. Low resolution mass spectra (LRMS) were recorded on an HP-5973 instrument (HP) and High resolution mass spectra (HRMS) were recorded on FTMS-7.0 instrument (Bruker) or IonSpec 4.7T instrument (Varian).

The purity of the final compounds was determined by HPLC on a DIONEX-P680 instrument (DIONEX) at 254 nm and all final compounds have a purity of >95%.

5.1. General procedure for preparation of compounds (*1a*–*d*)

The corresponding aldehyde (1 equiv), β-alanine (2 equiv), and **7** (or **8**) (2 equiv) were heated at 100 °C for 1 h in glacial acetic acid. Upon completion of the reaction, the mixture was cooled, the reaction was quenched with water, and the precipitate was filtered



Scheme 3. Reagents and conditions: (i) CS_2, Lac.sulfur, triethanolamine, water, reflux; (ii) chloroacetic acid, NaOAc, absolute alcohol, reflux; (iii) piperidine, absolute alcohol, 60 °C; (iv) $BrCH_2COOCH_3$, acetone, reflux; (v) K_2CO_3, water, methanol, reflux; (vi) β-alanine, acetic acid, reflux.

Table 1
Antibiofilm and anti-bacteria activity of thiazolidione derivatives.

Compound	Antibiofilm concentration(μM)	MIC(μM)
1a	>200	>200[a]
1b	25	6.25
1c	>200	>200
1d	>200	>200
1e	>200	>200
1f	>200	>200
1g	>200	>200
1h	>200	>200
1i	>200	>200
2a	50	25
2b	50	25
3a	25	12.5
3b	100	50
3c	6.25	6.25
3d	12.5	6.25
3e	25	12.5
3f	6.25	3.125
1	25	6.25

[a] The initiate dilution was 200 μM.

off. The solid products were filtered and recrystallized in methanol to obtain pure compound.

*5.1.1. 3-(5-((6-(Ethoxycarbonyl)-3-oxo-5,7-diphenyl-thiazolo[3,2-a] pyrimidin-2(5H)-ylidene)methyl) furan-2-yl)benzoic acid (**1a**)*

Prepared from compound **8** and 3-(5-formylfuran-2-yl)benzoic acid in 93% overall yield and obtained as a yellow solid. mp: 284–285 °C; IR(KBr): $\nu(cm^{-1})$: 3387, 3010, 1698, 1609, 1549, 1329, 1182, 1079; ^{1}H NMR (DMSO-*d6*) δ = 8.38(s, 1H, CH), 8.09–8.07 (d, J = 6.0 Hz, 1H), 7.97–7.95 (d, J = 6.0 Hz, 1H), 7.69–7.41 (m, 14H), 6.16 (s, 1H), 3.86–3.79(q, J = 6.9 Hz, 2H), 0.82–0.77 (t, J = 6.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ 166.7, 165.4, 163.9, 156.0, 155.9, 149.6, 149.1, 139.9, 138.5, 131.8, 129.5, 128.9, 128.8, 128.6, 128.2, 127.6, 127.3, 124.7, 121.6, 118.6, 116.6, 110.5, 109.9, 60.1, 55.7, 13.2; ESI-MS m/z 575.2 $[M - H]^+$; HRMS calcd for $C_{33}H_{25}N_2O_6S$ $[M + H]^+$ 577.1433, found 577.1430.

*5.1.2. 3-(5-((6-(Ethoxycarbonyl)-5-(4-methoxyphenyl)-3-oxo-7-phenyl- thiazolo[3,2-a]pyrimidin-2(5H)-ylidene)methyl)furan-2-yl) benzoic acid (**1b**)*

Prepared from compound **7** and 3-(5-formylfuran-2-yl)benzoic acid in 92% overall yield and obtained as a yellow solid. mp: 279–280 °C; IR(KBr), $\nu(cm^{-1})$: 3371, 3053, 1708, 1615, 1547, 1330, 1181, 1069; ^{1}H NMR (DMSO-*d6*) δ = 8.34 (s, 1H), 8.05–8.03 (d, J = 6.0 Hz, 1H), 7.93–7.91 (d, J = 6.0 Hz, 1H), 7.64–6.92 (m, 13H), 6.06 (s, 1H), 3.80–3.78 (q, J = 6.0 Hz, 2H), 3.69 (s, 3H), 0.79–0.75 (t, J = 6.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ 166.6, 165.4, 163.9, 159.4, 155.9, 155.6, 149.4, 149.1, 138.6, 131.9, 129.5, 128.9, 128.5, 128.2, 127.6, 124.7, 121.5, 118.5, 116.7, 114.1, 110.5, 110.1, 60.1, 55.1, 13.3; HRMS calcd for $C_{34}H_{27}N_2O_7S$ $[M + H]^+$ 607.1529, found 607.1533.

*5.1.3. Ethyl 2-((5-(3-(methoxycarbonyl)phenyl)furan-2-yl)methylene)-3-oxo-5,7-diphenyl- thiazolo[3,2-a]pyrimidine-6-carboxylate (**1c**)*

Prepared from compound **8** and 3-(5-formylfuran-2-yl)benzoate in 90% overall yield and obtained as a yellow solid. mp: 262–263 °C; IR(KBr), $\nu(cm^{-1})$: 3023, 1723, 1639, 1578, 1236, 1160, 933; ^{1}H NMR (DMSO-*d6*) δ 8.39 (s, 1H), 8.11 (d, J = 7.9 Hz, 1H), 7.97 (d, J = 7.8 Hz, 1H), 7.73–7.68 (m, 2H), 7.53–7.20 (m, 12H), 6.16 (d, J = 7.9 Hz, 1H), 3.90 (s, 3H), 3.83 (q, J = 7.0 Hz, 2H), 0.80 (t, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ 165.7, 165.4, 163.9, 155.8, 155.7, 149.5, 149.2, 139.8, 138.5, 130.6, 129.9, 129.2, 128.9, 128.7, 128.6, 127.7, 127.4, 124.6, 121.6, 118.6, 116.8, 110.8, 110.0, 60.1, 55.7, 52.4, 13.2; ESI-MS m/z 591.2 ($[M + H]^+$), 613.4($[M + Na]^+$); HRMS calcd for $C_{34}H_{27}N_2O_6S$ $[M + H]^+$ 591.1590, found 591.1586.

*5.1.4. Ethyl 2-((5-(3-(methoxycarbonyl)phenyl)furan-2-yl)methylene)-5-(4-methoxyphenyl)-3- oxo-7-phenyl-thiazolo[3,2-a]pyrimidine-6-carboxylate (**1d**)*

Prepared from compound **7** and methyl 3-(5-formylfuran-2-yl) benzoate in 90% overall yield and obtained as a yellow solid. mp: 248–249 °C; IR(KBr), $\nu(cm^{-1})$: 3030, 1715, 1548, 1244, 1175, 1031; ^{1}H NMR (DMSO-*d6*) δ 8.39 (s, 1H), 8.10 (d, J = 8.2 Hz, 1H), 7.96 (t, J = 8.1 Hz, 1H), 7.72–7.67 (m, 2H), 7.46–7.38 (m, 6H), 7.33 (d, J = 8.8 Hz, 2H), 7.28 (d, J = 3.7 Hz, 1H), 6.94 (d, J = 8.8 Hz, 2H), 6.11 (d, J = 7.1 Hz, 1H), 3.90 (s, 3H), 3.85–3.79 (m, 2H), 3.73 (s, 3H), 0.81 (t, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ 165.6, 165.4, 163.9, 159.4, 155.6, 149.2, 138.5, 131.9, 130.6, 129.8, 129.1, 128.8, 128.5, 128.2, 127.6, 124.6, 121.4, 118.4, 116.9, 114.1, 110.7, 110.1, 60.1, 55.1, 52.4, 13.2; HRMS calcd for $C_{35}H_{29}N_2O_7S$ $[M + H]^+$ 621.1697, found 621.1690.

*5.2. General procedure for preparation of compounds (**1e–j**)*

A mixture of corresponding benzaldehyde (3 mmol), **7** (or **9**) (3 mmol) in 15 mL ethanol in the presence of piperidine (5 mmol) was refluxed for 6 h. The mixture was cooled to room temperature and extracted with EtOAc. The organic layer was washed with brine, dried by anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by column chromatography (stationary phase, silica; mobile phase, petroleum ether/ethyl acetate 8/1).

*5.2.1. Ethyl 2-(3-hydroxybenzylidene)-5-(4-methoxyphenyl)-3-oxo-7-phenyl-thiazolo [3,2-a] pyrimidine-6-carboxylate (**1e**)*

Prepared from compound **7** and 3-hydroxybenzaldehyde in 90% overall yield and obtained as a yellow solid. mp: 187–188 °C; IR(KBr), $\nu(cm^{-1})$: 3403, 2992, 1700, 1609, 1541, 1327, 1244, 1175, 1061; ^{1}H NMR (DMSO-*d6*) δ 9.86 (s, 1H), 7.71 (s, 1H), 7.41 (d, J = 3.2 Hz, 5H), 7.33 (d, J = 8.8 Hz, 3H), 7.05 (d, J = 8.4 Hz, 1H), 6.98 (d, J = 1.8 Hz, 1H), 6.94 (d, J = 8.8 Hz, 2H), 6.89 (dd, J = 7.8, 2.0 Hz, 1H), 6.11 (s, 1H), 3.85–3.78 (m, 2H), 3.73 (s, 3H), 0.79 (t, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ 165.4, 164.2, 159.4, 157.8, 154.9, 149.1, 138.4, 133.9, 133.0, 131.7, 130.4, 128.9, 128.6, 128.1, 127.6, 121.1, 119.6, 118.0, 115.9, 114.1, 110.4, 60.1, 55.1, 13.2; HRMS calcd for $C_{29}H_{24}N_2O_5S$ (M^+) 512.1404, found 512.1406.

*5.2.2. Ethyl 2-(4-(dimethylamino)benzylidene)-5-(4-methoxyphenyl)-3-oxo-7-phenyl-thiazolo [3,2-a] pyrimidine-6-carboxylate (**1f**)*

Prepared from compound **7** and 4-(dimethylamino)benzaldehyde in 89% overall yield and obtained as a yellow solid. mp: 202–203 °C; IR(KBr), $\nu(cm^{-1})$: 2984, 1707, 1571, 1533, 1236, 1168, 1053; ^{1}H NMR (DMSO-*d6*) δ 7.68 (s, 1H), 7.44 (d, J = 9.0 Hz, 1H), 7.39 (s, 5H), 7.32 (d, J = 8.7 Hz, 2H), 6.93 (d, J = 8.7 Hz, 2H), 6.82 (d, J = 9.1 Hz, 2H), 6.11 (s, 1H), 3.86–3.77 (m, 2H), 3.73 (s, 3H), 3.02 (s, 6H), 0.80 (t, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ 165.5, 164.5, 159.3, 155.8, 151.6, 150.1, 138.8, 134.1, 132.2, 128.7, 128.5, 128.2, 127.6, 119.5, 114.1, 112.0, 111.6, 111.2, 109.4, 59.9, 55.0, 54.7, 13.2; HRMS calcd for $C_{31}H_{29}N_3O_4S$ (M^+) 539.1880, found 539.1879.

*5.2.3. Ethyl 2-(3-nitrobenzylidene)-5-(4-methoxyphenyl)-3-oxo-7-phenyl- thiazolo[3,2-a]pyrimidine-6-carboxylate (**1g**)*

Prepared from compound **7** and 3-nitrobenzaldehyde in 90% overall yield and obtained as a yellow solid. mp: 144–145 °C; IR (KBr), $\nu(cm^{-1})$: 2984, 1693, 1541, 1335, 1244, 1191, 1138, 1061; ^{1}H NMR (DMSO-*d6*) δ 7.98 (s, 1H), 7.45–7.37 (m, 6H), 7.36–7.32 (m, 2H), 7.32–7.26 (m, 2H), 7.00–6.92 (m, 2H), 6.13 (s, 1H), 3.86–3.79 (m, 2H), 3.74 (s, 3H), 0.80 (t, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ 165.4, 163.8, 159.5, 154.2, 148.7, 148.2, 138.2, 135.1,

134.5, 131.4, 130.9, 130.3, 129.4, 128.2, 127.9, 124.5, 114.0, 110.8, 60.2, 55.3, 55.1, 13.2; HRMS calcd for $C_{31}H_{29}N_3O_4S$ (M^+) 541.1307, found 541.1308.

*5.2.4. Ethyl 2-(4-hydroxy-3-methoxybenzylidene)-5-(4-methoxyph enyl)-3-oxo-7-phenyl- thiazolo [3,2-a] pyrimidine-6-carboxylate (**1h**)*

Prepared from compound **7** and 4-hydroxy-3-methoxybenzalde hyde in 85% overall yield and obtained as a yellow solid. mp: 153–154 °C; IR(KBr), ν(cm^{-1}): 3418, 2984, 1707, 1541, 1502, 1236, 1175, 1061; ^{1}H NMR (DMSO-*d6*) δ 10.00 (s, 1H), 7.73 (s, 1H), 7.41 (s, 5H), 7.33 (d, J = 8.8 Hz, 2H), 7.17 (d, J = 2.0 Hz, 1H), 7.09 (dd, 1H), 6.98–6.91 (m, 3H), 6.12 (s, 1H), 3.86–3.78 (m, 5H), 3.73 (s, 3H), 0.80 (t, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ 165.4, 164.4, 159.4, 155.4, 149.8, 149.5, 148.0, 138.5, 133.7, 131.9, 128.7, 128.5, 128.2, 127.6, 124.2, 116.2, 115.5, 114.0, 109.9, 60.0, 55.6, 55.0, 54.9, 13.2; ESI-MS m/z 543.1([M + H]$^+$), 565.3 ([M + Na]$^+$); HRMS calcd for $C_{33}H_{27}N_2O_6S$ [M + H]$^+$ 543.1569, found 543.1584.

*5.2.5. Ethyl 2-(4-hydroxybenzylidene)-5-(4-methoxyphenyl)-3-oxo -7-phenyl- thiazolo[3,2-a]pyrimidine-6-carboxylate (**1i**)*

Prepared from compound **7** and 4-hydroxybenzaldehyde in 87% overall yield and obtained as a yellow solid. mp: 150–151 °C; IR (KBr), ν(cm^{-1}): 3418, 2904, 1700, 1548, 1510, 1235, 1160, 1053; ^{1}H NMR (DMSO-*d6*) δ 10.35 (s, 1H), 7.72 (s, 1H), 7.51–7.46 (m, 2H), 7.42–7.38 (m, 5H), 7.35–7.29 (m, 2H), 6.97–6.89 (m, 4H), 6.11 (s, 1H), 3.86–3.77 (m, 2H), 3.72 (s, 5H), 0.80 (t, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ 165.4, 164.5, 160.2, 159.4, 155.4, 149.6, 138.6, 133.4, 132.4, 132.0, 128.8, 128.5, 128.2, 127.6, 123.7, 116.4, 115.8, 115.2, 114.1, 110.0, 60.0, 55.0, 54.9, 13.2; ESI-MS m/z 513.1 ([M + H]$^+$), 535.0([M + Na]$^+$); HRMS calcd for $C_{29}H_{25}N_2O_5S$ [M + H]$^+$ 513.1487, found 513.1478.

*5.2.6. Ethyl 2-(4-hydroxybenzylidene)-5-(benzo[d][1,3]dioxol-5-yl)-3-oxo-7-phenyl-thiazolo [3,2-a] pyrimidine-6-carboxylate (**1j**)*

Prepared from compound **9** and 4-hydroxybenzaldehyde in 85% overall yield and obtained as a yellow solid. mp: 160–161 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.02 (s, 1H), 7.38–7.00 (m, 12H), 6.23 (s, 1H), 5.92 (s, 2H), 3.94–3.87 (q, J = 6.9 Hz, 2H), 0.90–0.86 (t, J = 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 166.3, 165.7, 158.9, 157.2, 151.5, 148.3, 148.2, 139.0, 134.5, 133.7, 132.6, 129.1, 128.4, 128.2, 125.5, 122.2, 116.7, 110.3, 108.6, 101.5, 61.0, 56.0, 13.7; ESI-MS m/z 527.1([M + H]$^+$), 549.2([M + Na]$^+$).

*5.3. General procedure for preparation of compounds (**2a**, **2b**)*

A mixture of compound **1i** or **1j** (1 mmol), methyl bromoacetate (1.5 mmol) and potassium carbonate (1.5 mmol) in acetone (15 ml) was refluxed for 4 h. The solvent was evaporated under reduced pressure and the solid residue was purified by silica gel column chromatography using petroleum ether/ethyl acetate (4:1 v/v) as mobile phase. The purified solid (1 mmol) and potassium carbonate (1 mmol) in the medium of 30 ml methanol and 20 ml water was refluxed for 12 h. The reaction mixture was then filtered and the filtrate was acidified with HCl until solid precipitated. The solid products were filtered and recrystallized in methanol to obtain pure compound **2a** or **2b**.

*5.3.1. 2-(4-((6-(Ethoxycarbonyl)-5-(4-methoxyphenyl)-3-oxo-7-phenyl-thiazolo [3,2-a] pyrimidin-2(5H)-ylidene)methyl)phenoxy) acetic acid (**2a**)*

Prepared from compound **1i** in overall yield 71% and obtained as a yellow solid. mp: 185–186 °C; IR(KBr), ν(cm^{-1}): 3334, 2984, 1715, 1548, 1502, 1244, 1175, 1069; ^{1}H NMR (300 MHz, DMSO-*d6*) δ = 7.73 (s, 1H), 7.62–6.93(m, 13H), 6.20(s, 1H), 4.85(s, 1H), 3.87–3.84 (q, J = 3.0 Hz, 2H), 3.76(s, 3H), 0.86–0.82(t, J = 6.0 Hz, 3H); ^{13}C NMR (DMSO-*d6*) δ 165.5, 164.4, 163.1, 160.1, 159.4, 155.2, 149.52, 138.6, 132.9, 131.9, 131.5, 128.9, 128.6, 128.2, 127.6, 125.4, 116.6, 115.5, 115.0, 114.1, 110.1, 60.1, 55.1, 13.3; ESI-MS m/z 571.1([M + H]$^+$), 593.1 ([M + Na]$^+$); HRMS calcd for $C_{31}H_{27}N_2O_7S$ [M + H]$^+$ 571.1539, found 571.1533.

*5.3.2. 2-(4-((5-(Benzo[d][1,3]dioxol-5-yl)-6-(ethoxycarbonyl)-3-oxo -7-phenyl-thiazolo[3,2-a]pyrimidin-2(5H)-ylidene)methyl)phenoxy) acetic acid (**2b**)*

Prepared from compound **1j** in overall yield 65% and obtained as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d6) δ = 7.79(s, 1H), 7.59–6.89(m, 12H), 6.08(s, 1H), 6.02(s, 2H), 4.78(s, 2H), 3.86–3.79 (q, J = 6.9 Hz, 2H), 0.82–0.78(t, J = 6.0 Hz, 3H); ESI-MS m/z 585.4 ([M + H]$^+$),583.5([M − H]$^+$).

*5.4. 5-(4-Hydroxybenzylidene)-3-(4-methoxybenzyl)-2-(4-methox ybenzylimino)thiazolidin-4-one (**14**)*

A mixture of 4-hydroxybenzaldehyde (3 mmol), compound **10** (3 mmol) in 15 mL ethanol in the presence of piperidine (5 mmol) was refluxed for 6 h. Upon completion of the reaction, the reaction mixture was extracted with EtOAc. The organic layer was washed with brine, dried by anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography using petroleum ether/ethyl acetate (4:1 v/v) as mobile phase to obtain pure compound **14** as a yellow solid: yield, 95%; ^{1}H NMR ($CDCl_3$) δ = 7.69 (s, 1H), 7.44–6.80 (m, 12H), 5.05 (s, 2H), 4.58 (s, 2H), 3.81 (s, 3H), 3.77 (s, 3H); ESI-MS m/z 461.1 ([M + H]$^+$), 483.2([M + Na]$^+$).

*5.5. 2-(4-((3-(4-Methoxybenzyl)-2-(4-methoxybenzylimino)-4-oxo thiazolidin-5-ylidene)methyl) phenoxy)acetic acid (**3a**)*

A mixture of compound **14** (1 mmol), methyl bromoacetate (1.5 mmol) and potassium carbonate (1.5 mmol) in acetone (15 ml) was refluxed for 6 h. The solvent was evaporated under reduced pressure and the solid residue was purified by silica gel column chromatography using petroleum ether/ethyl acetate (4:1 v/v) as mobile phase. The purified solid (1 mmol) and potassium carbonate (2 mmol) in the medium of 25 ml methanol and 15 ml water was refluxed for 12 h. The reaction mixture was then filtered and the filtrate was acidified with HCl until solid precipitated. The solid products were filtered and recrystallized in methanol to obtain pure compound as a yellow solid: yield, 70%; mp: 196–197 °C; IR(KBr), ν (cm^{-1}): 3327, 2992, 1746, 1685, 1647, 1510, 1244; ^{1}H NMR (DMSO-*d6*) δ = 7.70 (s, 1H), 7.62–6.85 (m, 12H), 4.92 (s, 2H), 4.76 (s, 2H), 4.54 (s, 2H), 3.73 (s, 3H), 3.71 (s, 3H); ^{13}C NMR (DMSO-*d6*) δ 169.7, 165.8, 159.0, 158.6, 158.1, 148.0, 131.7, 130.9, 129.4, 129.3, 128.4, 126.3, 118.3, 115.3, 113.7, 113.7, 64.5, 55.0, 54.9, 45.0; ESI-MS m/z 519.0([M + H]$^+$); HRMS calcd for $C_{28}H_{27}N_2O_6S$ [M + H]$^+$ 519.1590, found 519.1591.

*5.6. The synthesis of 3-(5-((3-(4-methoxybenzyl)-2-(4-methoxyben zylimino)-4-oxothiazolidin-5-ylidene)methyl) furan-2-yl) benzoic acid (**3b**) illustrates the general sequence used to prepare compounds **3b–f***

*5.6.1. 3-(5-((3-(4-Methoxybenzyl)-2-(4-methoxybenzylimino)-4-oxothiazolidin-5-ylidene)methyl) furan-2-yl)benzoic acid (**3b**)*

A mixture of methyl 3-(5-formylfuran-2-yl)benzoate (3 mmol), β-alanine (3 mmol), and compound **10** (3 mmol)were heated at 100 °C for 1 h in acetic acid (10 mL). Upon completion of the reaction, the mixture was cooled, the reaction was quenched with water (20 mL), and the precipitate was filtered off. The solid was washed with water and methanol and then finally dried with diethyl ether to give the desired compound as a yellow solid: yield, 92%; mp: 245–246 °C; IR

(KBr), $\nu(cm^{-1})$: 3342, 2993, 1693, 1638, 1502, 1252; ^1H NMR (DMSO-*d6*) δ = 8.45 (s, 1H), 8.05–8.03 (d, J = 6.0 Hz, 1H), 7.95–7.93 (d, J = 6.0 Hz, 1H), 7.63–6.87 (m, 12H), 4.92 (s, 2H), 4.62 (s, 2H), 3.72 (s, 6H); ^{13}C NMR (DMSO-*d6*) δ 166.8, 165.3, 158.6, 158.1, 155.0, 149.5, 148.8, 131.7, 131.0, 129.5, 129.3, 129.1, 128.6, 128.4, 128.0, 124.6, 119.8, 118.5, 115.7, 113.7, 110.0, 55.0, 54.6, 44.8; ESI-MS m/z 555.1($[M + H]^+$); HRMS calcd for $C_{31}H_{27}N_2O_6S$ $[M + H]^+$ 555.1590, found 555.1585.

*5.6.2. 3-(5-((3-(4-Methoxyphenyl)-2-(4-methoxyphenylimino) -4-oxothiazolidin-5-ylidene)methyl) furan-2-yl)benzoic acid (**3c**)*

Prepared from methyl 3-(5-formylfuran-2-yl)benzoate and com pound **11** and obtained as a yellow solid: yield, 90%; mp: 219–220 °C; IR(KBr), $\nu(cm^{-1})$: 3302, 2984, 1700, 1616, 1510, 1244; ^1H NMR (DMSO-*d6*) δ = 8.30(s, 1H), 7.94–7.92(d, J = 6.0 Hz, 1H), 7.89–7.87(d, J = 6.0 Hz, 1H), 7.64–6.97(m, 12H), 3.82(s, 3H), 3.79 (s, 3H); ^{13}C NMR (DMSO-*d6*) δ 166.7, 165.3, 159.1, 156.5, 155.0, 150.8, 149.7, 140.7, 131.8, 129.6, 129.2, 127.7, 124.5, 122.2, 119.5, 119.0, 116.2, 114.5, 114.1, 110.2, 55.4, 55.3; ESI-MS m/z 525.3($[M - H]^+$); HRMS calcd for $C_{29}H_{23}N_2O_6S$ $[M + H]^+$ 527.1277, found 527.1275.

*5.6.3. 3-(5-((4-Oxo-3-(4-methylphenyl)-2-(4-methylphenylimino) thiazolidin-5-ylidene)methyl) furan-2-yl)benzoic acid (**3d**)*

Prepared from methyl 3-(5-formylfuran-2-yl)benzoate and com pound **12** and obtained as a yellow solid: yield, 93%, mp: 259–260 °C; IR(KBr), $\nu(cm^{-1})$: 3395, 3038, 1715, 1624, 1510, 1373, 1168; ^1H NMR (DMSO-*d6*) δ = 13.18(s, 1H), 8.27(s, 1H), 7.94–7.92(d, J = 6.0 Hz, 1H), 7.87–7.84(d, J = 6.0 Hz, 1H), 7.64–6.91(m, 12H), 2.38(s, 3H), 2.34(s, 3H); ^{13}C NMR (DMSO-*d6*) δ 166.7, 165.2, 155.1, 151.0, 149.7, 145.2, 138.1, 133.9, 132.4, 131.8, 129.7, 129.3, 128.2, 127.7, 124.5, 120.8, 119.6, 118.9, 116.3, 110.3, 20.7, 20.5; ESI-MS m/z 493.3($[M - H]^+$); HRMS calcd for $C_{29}H_{23}N_2O_4S$ $[M + H]^+$ 495.1379, found 495.1382.

*5.6.4. 3-(5-((4-Oxo-3-(2-methylphenyl)-2-(2-methylphenylimino) thiazolidin-5-ylidene)methyl) furan-2-yl)benzoic acid (**3e**)*

Prepared from methyl 3-(5-formylfuran-2-yl)benzoate and como-pund **13** and obtained as a yellow solid: yield, 90%, mp: 262–263 °C; IR (KBr), $\nu(cm^{-1})$: 3380, 2984, 1700, 1639, 1541, 1373, 1161; ^1H NMR (DMSO-*d6*) δ = 8.23 (s, 1H), 7.93–7.91 (d, J = 6.0 Hz, 1H), 7.83–7.81 (d, J = 6.0 Hz, 1H), 7.69–6.96 (m, 12H), 2.25 (s, 3H), 2.05 (s, 3H); ^{13}C NMR (DMSO-*d6*) δ 166.7, 165.0, 155.2, 150.2, 149.6, 146.4, 136.0, 134.3, 131.8, 130.6, 129.2, 127.7, 126.9, 126.7, 124.8, 124.5, 119.9, 119.6, 118.7, 116.7, 110.3, 17.3, 17.0; ESI-MS m/z 495.0($[M + H]^+$),517.1($[M + Na]^+$); HRMS calcd for $C_{29}H_{23}N_2O_4S$ $[M + H]^+$ 495.1372, found 495.1373.

*5.6.5. 4-(5-((4-Oxo-3-(2-methylphenyl)-2-(2-methylphenylimino) thiazolidin-5-ylidene)methyl) furan-2-yl)benzoic acid (**3f**)*

Prepared from methyl 4-(5-formylfuran-2-yl)benzoate and com pound **11** and obtained as a yellow solid: yield, 91%, mp: 289–290 °C; IR (KBr), $\nu(cm^{-1})$: 3382, 2980, 1693, 1601, 1358, 1282, 1153; ^1H NMR (DMSO-*d6*) δ = 7.69(s, 1H), 7.91–7.90(d, J = 6.0 Hz, 1H), 7.72–7.71(d, J = 6.0 Hz, 1H), 7.47–6.97(m, 12H), 2.25(s, 3H), 2.07(s, 3H); ^{13}C NMR (DMSO-*d6*) δ 166.6, 165.0, 154.9, 150.7, 150.0, 146.8, 136.0, 134.2, 132.4, 130.6, 130.2, 129.8, 129.2, 126.9, 126.5, 124.8, 123.6, 119.8, 119.8, 116.5, 111.4, 17.4, 17.1; ESI-MS m/z 493.3($[M - H]^+$); HRMS calcd for $C_{29}H_{23}N_2O_4S$ $[M + H]^+$ 495.1379, found 495.1375.

5.7. Antibiofilm and antibacterial activity assay

The *S. epidermidis* ATCC35984 was selected as the biofilm-forming bacteria employed in the study because *S. epidermidis* is one of the most frequent causes of infections in connection with surgically implanted medical devices [19,20]. In the antibiofilm concentration experiment, an overnight culture of *S. epidermidis* strain ATCC35984 was diluted 1:200 in TSB containing 0.25% glucose, and then 200 μl bacterial suspension was added into the wells of sterile 96-well polystyrene microtiter plates (Falcon) incubated at 37 °C for 6 h. The plates with young biofilm were washed gently four times with sterile PBS before adding fresh TSB containing the various derivatives at gradient dilution, and incubated at 37 °C for 18 h. The plates were washed again four times with PBS, and 99% methanol was added for 15 min. After air-dried, the biofilm was dyed by crystal violet. A well with young biofilm washed by sterile PBS then dried was served as negative control; this value was subtracted from the experimental readings. Another well with young biofilm added sterile TSB was served as positive control. Each assay was performed in triplicate. The experiment was repeated three times. MIC assays for the antibacterial activities of the compounds were performed according to the broth micro-dilution (in tubes) method of the Clinical and Laboratory Standards Institute (CLSI) of America [21].

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