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Synthesis and antibiofilm activity of marine natural product-based 4-thiazolidinones derivatives

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ABSTRACT

4-Thiazolidinones derivatives of marine bromopyrrole alkaloids were synthesized as potential antibiofilm compounds. Among the synthesized compounds, some showed promising antibiofilm activity. Biological data revealed that 1,3-thiazolidin-4-one derivatives are more potent antibiofilm agents compared to 1,3-thiazinan-4-ones. Antibiofilm activity of compound **4b**, **4c** (MIC = 0.78 μg/ml) was 3-fold superior than standard vancomycin (MIC = 3.125 μg/ml) while activity of compound **4d**, **4f**, **4g** and **4h** was 2-fold (MIC = 1.56 μg/ml) against *Staphylococcus aureus* biofilm. Compound **4b–4h** showed equal antibiofilm activity against *Staphylococcus epidermidis* compared to standard Vancomycin (MIC = 3.125 μg/ml).

Nosocomial infections occur worldwide, both in the developed and developing world. They are a significant burden to patients and public health. Coagulase-negative staphylococci and *Staphylococcus aureus* have emerged as predominant cause of hospital acquired infections and can induce a wide spectrum of diseases associated with morbidity and mortality. Patients at risk include those with prosthetic devices, intravascular catheters, or other foreign bodies in place, and immunocompromised hosts.[1] On account of the inevitable development of resistance, the discovery of new antimicrobial agents active against not only planktonic bacteria but also against biofilms that are intrinsically resistant to conventional antibiotics represents an important task.[2–5]

One approach is to improve the activity of natural anti-microbial substances by synthesis of analog compounds. For this reason, we turned our attention to naturally produced organohalogens.[6] Our interest in halogenated pyrrole derivatives led to synthesis and antimicrobial evaluation of few analogues of pyoluteorin.[7] In continuation to this we found that bromopyrrole alkaloids; a family of marine alkaloids represents a fascinating example of the large variety of secondary metabolites formed by marine sponges. These compounds are involved in the sponge's defense mechanism against fishes. Also, several pharmacologically important bromopyrrole congeners have been previously described as having antihistaminic, antiserotonergic and antineoplastic activity. Furthermore, these natural products also possess antibacterial, antifungal and anitibiofilm activity.[8–10] Most of these compounds are defined by the signature of bromopyrrole carboxamide with oroidin as there prototype alkaloid reported for its antibiofilm activity.[8] Structure–activity relationship performed on synthetic library of oroidin derivatives indicated that N-methylation of the pyrrole ring led to increased antibiofilm activity against medically relevant Gram-negative γ-proteobacterium *Pseudomonas aeruginosa*, as indicated by the most active member of the library, dihydrosventrin (DHS)[11] (Fig. 1).

The structural and therapeutic diversity coupled with commercial viability of small heterocyclic molecules has fascinated organic and medicinal chemists. In recent years, 4-thiazolidinones are the most extensively investigated class of compounds. 4-Thiazolidinones have many interesting activity profiles namely COX-1 inhibitors,[12] non-nucleoside inhibitors of HIV-RT,[13] inhibitors of aldose reductase,[14,15] antidiabetic,[16] inhibitors of the bacterial enzyme MurB and YycG histidine kinase.[17,18] Moreover derivatives of 4-thiazolidinones have been reported for antitubercular, antifungal and anthelmintic activities.[19] Keeping this in a view, 4,5-dibromopyrrole carboxamide moiety was incorporated in to 4-thiazolodinone and evaluated for antibiofilm activity. We also investigated effect of replacement of five member 1,3-thiazolidin-4-one by six member 1,3-thiazinan-4-one ring and systematic modification of the substituent on the aromatic ring attached to 4-thiazolidinone ring. (Fig. 1)

The target compounds were synthesized as illustrated in Schemes 1 and 2. Trichloroacetylation of respective pyrroles gave an excellent yield of **1**.[20] Compound **1** was brominated using bromine in chloroform to give **2**.[21] Compound **2** was further converted into corresponding hydrazides **3** by stirring with hydrazine hydrate at room temperature (Scheme 1). The desired 1,3-thiazolidin-4-ones /1,3-thiazinan-4-ones were synthesized in the quantitative yield by one-pot condensation of amine, benzaldehyde, mercaptoacetic/mercaptopropinoic acid in the ratio 1:2:3. Compound **3**

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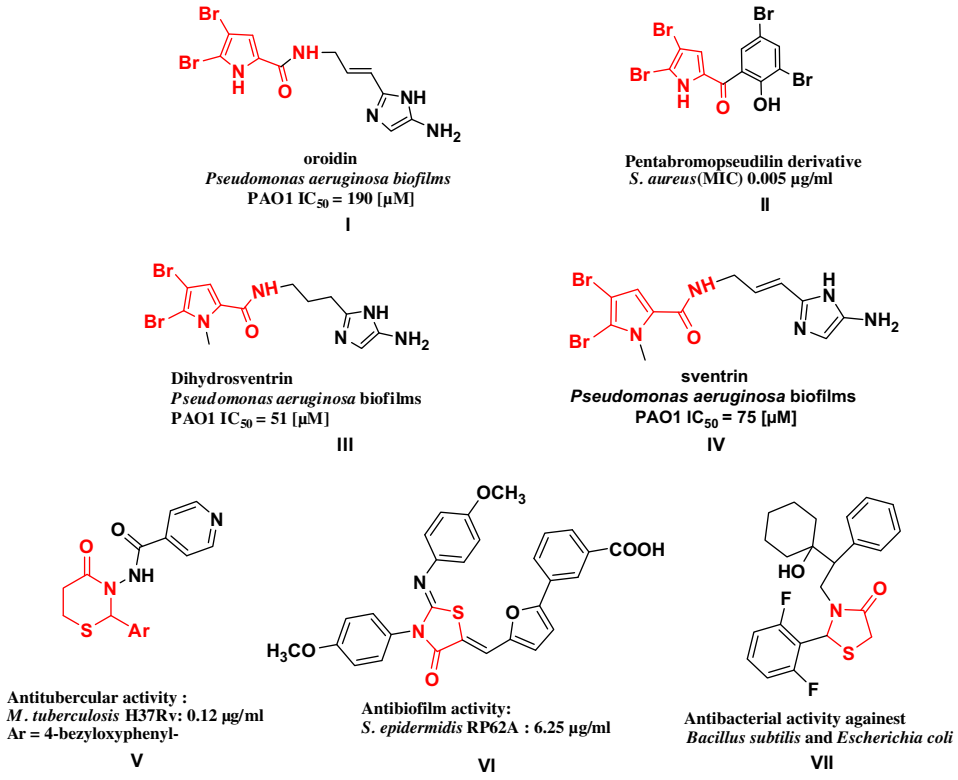
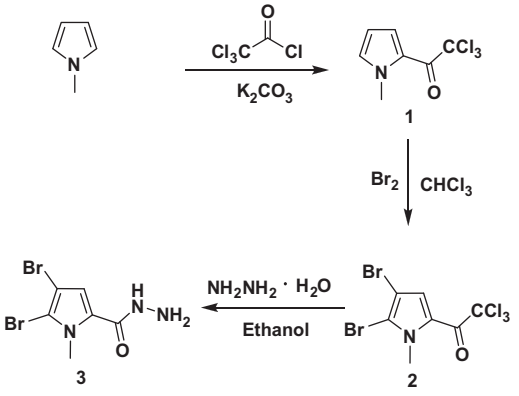


Figure 1. Reported bromopyrrole alkaloids and 4-thiazolidinones with antibacterial and antibiofilm activity.



Scheme 1. Synthesis of 4,5-dibromo-1-methyl-1*H*-pyrrole-2-carbohydrazide.

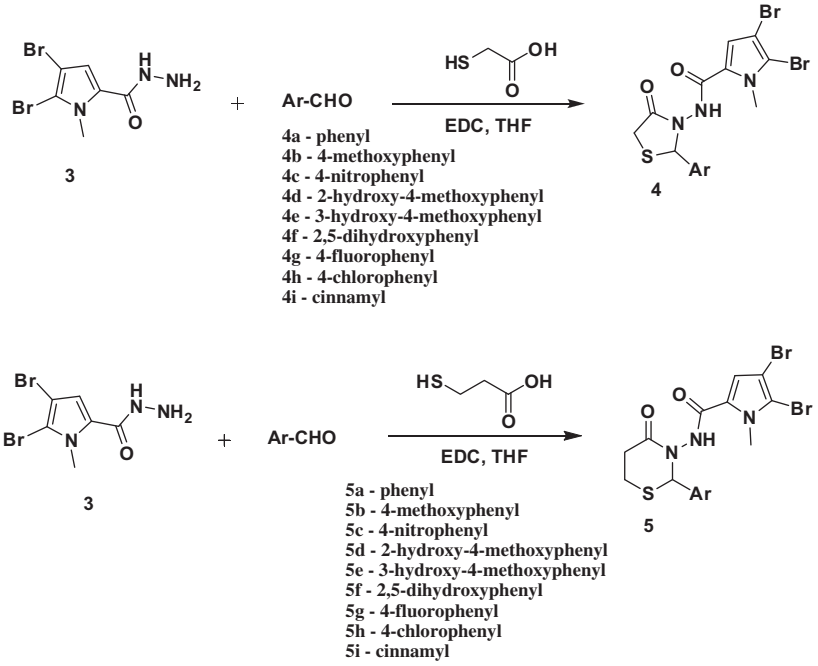
was dissolved in THF and the reaction mixture was cooled to 0 °C, the corresponding aldehyde was then added drop wise, followed by mercaptoacetic acid/3-mercaptopropionic acid and 1.2 equiv of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, the reaction mixture was then slowly warmed to room temperature and stirred at room temperature for another 5–6 h (monitored by TLC for completion), it was then quenched with water and worked up to give the desired 1,3-thiazolidin-4-one/1,3-thiazinan-4-one in quantitative yield. (Scheme 2)[22,23]

The spectral data (IR, ^{1}H NMR and MS) of all synthesized compounds were in agreement with the proposed structures. The MS of all the compounds exhibited the (M+) as molecular ion peak, confirming the molecular weight. Presence of bromo was confirmed with the presence of [M+2], [M+4] peaks in mass spectra. The ^{1}H NMR spectra of all compounds showed single at δ 4.0–3.8 corresponding to N–CH_3 attached to pyrrole core while the amide N–H proton resonates between δ 8.4 and 8.1. Proton at third position of pyrrole ring resonates between δ 6.6 and 6.4. The proton of substituted aryl ring resonates between 7.9 and 6.4.

The newly prepared compounds were screened for their minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and antibiofilm concentration assay against *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC29213 and *Enterococcus faecalis* ATCC 29212 bacterium using a standard tube-dilution assay (Table 1). MIC assays for the antibacterial activities of the compounds were performed according to the broth microdilution (in tubes) method of the Clinical and Laboratory Standards Institute (CLSI) of America.[24] MBC of the compounds was obtained by sub-culturing 100 μl from each negative (no visible bacterial growth) tube from the MIC assay, onto substance-free Mueller–Hinton agar plates. The plates were incubated at 37 °C for 24 h, and the MBC was defined as the lowest concentration of compounds which produced subcultures growing no more than five colonies on each plate.

In the antibiofilm concentration experiment, overnight culture of bacterium (*Staphylococcus aureus* ATCC 29213/*Staphylococcus epidermidis* ATCC 12228/*Enterococcus faecalis* ATCC 29212) was diluted 1:10 in TSB (OD 600 = 0.6–0.8), then was diluted 1:200 in MH. The bacterial suspension was inoculated 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 concentration compounds, and incubated at 37 °C for 16 h. The initiate dilution was 200 μM. For all assay standard used was Vancomycin.[25]

The synthesized series of compounds showed promising activity against *S. aureus, S. epidermidis* and *E. faecalis.* As indicated in Table 1, most of synthesized compounds generally showed potent to moderate antibacterial and antibiofilm activity against activity all tested gram-positive bacterium. Antibiofilm activity of compound **4b** and **4c** (MIC = 0.78 μg/ml) was 3-fold superior to



Scheme 2. Synthesis of 1,3-thiazolidin-4-one **4** and 1,3-thiazinan-4-one **5**.

Table 1
Antibacterial and antibiofilm test results: (MIC μg/ml) [a,b]

S. No.	Ar	*S. aureus* MBC (MIC) μg/mL	*S.epidermidis* MBC (MIC) μg/mL	*E. faecalis* MBC(MIC) μg/mL	*S. aureus* antibiofilm concentration (μg/mL)	*S. epidermidis* antibiofilm concentration (μg/mL)	*E. faecalis* antibiofilm concentration (μg/mL)
4a	Phenyl	6.25 (1.56)	6.25 (3.125)	25 (12.5)	3.125	12.5	12.5
4b	4-Methoxyphenyl	6.25 (0.78)	3.125 (1.56)	25 (6.25)	0.78	3.125	6.25
4c	4-Nitrophenyl	6.25 (0.78)	6.25 (1.56)	25 (12.5)	0.78	3.125	12.5
4d	2-Hydroxy-4-methoxyphenyl	6.25 (0.78)	3.125 (1.56)	25 (12.5)	1.56	3.125	12.5
4e	3-Hydroxy-4-methoxyphenyl	6.25 (1.56)	6.25 (1.56)	12.5 (3.125)	3.125	3.125	12.5
4f	2,5-Dihydroxyphenyl	3.125 (0.78)	3.125 (1.56)	12.5 (6.25)	1.56	3.125	12.5
4g	4-Fluorophenyl	3.125 (0.78)	3.125 (1.56)	12.5 (3.12)	1.56	3.125	12.5
4h	4-Chlorophenyl	3.125 (0.78)	3.125 (1.56)	25 (12.5)	1.56	3.125	12.5
4i	Cinnamyl	12.5 (6.25)	12.5 (25)	12.5 (25)	6.125	6.25	6.25
5a	Phenyl	6.25 (12.5)	12.5 (6.25)	100 (50)	12.5	12.5	25
5b	4-Methoxyphenyl	6.25 (3.125)	12.5 (6.25)	100 (50)	6.25	12.5	25
5c	4-Nitrophenyl	6.25 (3.125)	12.5 (6.25)	50(50)	6.25	12.5	25
5d	2-Hydroxy-4-methoxyphenyl	6.25 (3.125)	12.5 (6.25)	50 (25)	6.25	12.5	25
5e	3-Hydroxy-4-methoxyphenyl	6.25 (3.125)	12.5 (6.25)	50 (25)	6.25	12.5	25
5f	2,5-Dihydroxyphenyl	12.5 (3.125)	12.5 (6.25)	50 (25)	6.25	12.5	25
5g	4-Fluorophenyl	12.5 (3.125)	12.5 (6.25)	50 (25)	6.25	12.5	25
5h	4-Chlorophenyl	12.5 (3.125)	12.5 (6.25)	50 (25)	6.25	12.5	25
5i	Cinnamyl	50 (12.5)	12.5 (6.25)	50 (25)	25	25	50
Standard[c]		3.125 (1.56)	3.125 (1.56)	6.25 (3.125)	3.125	3.125	3.125

[a] The initiate dilution was 200 μM.
[b] The MIC values are given in bracket and MBC out of bracket.
[c] For all assay standard used was Vancomycin.

that of standard Vancomycin (MIC = 3.125 μg/ml) while activity of compound **4d**, **4f**, **4g** and **4h** was 2-fold (MIC = 1.56 μg/ml) to that of standard against *S. aureus*. Compound **4b–4h** showed equal antibiofilm activity against *S. epidermidis* compared to standard Vancomycin (MIC = 3.125 μg/ml). All 1,3-thiazolidin-4-one derivatives (**4a–4i**) showed moderate antibiofilm activity against *E. faecalis* (MIC = 6.25–12.5 μg/ml) compared to standard Vancomycin. Better antibiofilm and antibacterial activities shown by **4a–4i** in comparison to **5a–5i** against all tested bacterial pathogens indicated that replacement of five member 1,3-thiazolidin-4-one ring by six member 1,3-thiazinan-4-one leads to decrease in antibacterial and antibiofilm activity. Biological activities shown by derivatives **4i** and **5i** revealed that replacement of aryl ring by cinnamyl moiety lead to decrease in antibacterial and antibiofilm activities.

In conclusion, 18 new 4-thiazolidinone derivatives of marine bromopyrrole alkaloids were synthesized and evaluated for their

antibacterial and antibiofilm activity. The synthesized hybrids showed promising activity against *S. aureus* and *S. epidermidis*. Biological data revealed that 1,3-thiazolidin-4-one derivatives are more potent antibiofilm agents in comparison to 1,3-thiazinan-4-ones. Compound **4b–4h** showed equal antibiofilm activity against *S. epidermidis* compared to standard Vancomycin (MIC = 3.125 μg/ml). The antibiofilm activity at concentrations as low as 0.78 μg/ml shown by compounds **4b** and **4c** and 1.56 μg/ml by compound **4d**, **4f**, **4g** and **4h** against *S. aureus* indicates that these compounds can act as leads for development of newer antibiofilm agent. Further studies on these compounds and optimization of their structures leading to novel analogues with superior biological properties are ongoing in our laboratories.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.09.073.

References and notes

1. Costerton, J. W.; Lewandowski, Z.; Caldwell, D. E.; Korber, D. R.; Lappinscott, H. M. *Annu. Rev. Microbiol.* **1995**, *49*, 711.
2. Beech, I. B.; Sunner, J. A.; Hiraoka, K. *Int. Microbiol.* **2005**, *8*, 157.
3. Flemming, H. C.; Griebe, T.; Schaule, G. *Water Sci. Technol.* **1996**, *34*, 517.
4. Lahtinen, T.; Kosonen, M.; Tiirola, M.; Vuento, M.; Oker-Blom, C. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 734.
5. Rodrigues, C.; Takita, M.; Coletta-Filho, H.; Olivato, J.; Caserta, R.; Machado, M.; De Souza, A. *Appl. Microbiol. Biotechnol.* **2008**, *77*, 1145.
6. Gribble, G. W. *Chemosphere* **2003**, *52*, 289.
7. Rane, R. A.; Telvekar, V. N. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5681.
8. Aiello, A.; Fattorusso, E.; Menna, M.; Taglialatela-Scafati, O. *Modern Alkaloids Structure, Isolation, Synthesis and Biology*; Wiley-VCH, 2008, p. 271.
9. Tasdemir, D.; Topaloglu, B.; Perozzo, R.; Brun, R.; O'Neill, R.; Carballeira, N. M.; Zhang, X.; Tonge, P. J.; Linden, A.; Ruedi, P. *Bioorg. Med. Chem.* **2007**, *15*, 6834.
10. Xiong, S.; Pang, H.; Fan, J.; Ge, F.; Yang, X.; Liu, Q.; Liao, X.; Xu, S. *J. Pharmacol.* **2010**, *159*, 909.
11. Richards, J.; Ballard, T.; Huigens, R.; Melander, C. *ChemBioChem* **2008**, *9*, 1267.
12. Look, G. C.; Schullek, J. R.; Homes, C. P.; Chinn, J. P.; Gordon, E. M.; Gallop, M. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 707.
13. Barreca, M. L.; Chimirri, A.; Luca, L. D.; Monforte, A.; Monforte, P.; Rao, A.; Zappala, M.; Balzarini, J.; De Clercq, E.; Pannecouque, C.; Witvrouw, M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1793.
14. Maccari, R.; Corso, A. D.; Giglio, M.; Moschini, R.; Mura, U.; Ottana, R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 200.
15. Ottana, A.; Maccari, R.; Giglio, M.; Corso, A. D.; Cappiello, M.; Mura, U.; Cosconati, S.; Marinelli, M.; Novellino, E.; Sartini, S.; La-Motta, C.; Settimo, F. D. *Eur. J. Med. Chem.* **2011**, *46*, 2797.
16. Kini, D.; Ghate, M. *Eur. J. Chem.* **2011**, *8*, 386.
17. Anders, C. J.; Bronson, J. J.; D'Andrea, S. V.; Deshpande, S. M.; Falk, P. J.; Grant-Young, K. A.; Harte, W. E.; Ho, H.; Misco, P. F.; Robertson, J. G.; Stock, D.; Sun, Y.; Walsh, A. W. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 715.
18. Schreiber, M.; Res, J.; Matter, A. *Curr. Opin. Cell Biol.* **2009**, *21*, 325.
19. Jain, A. K.; Vaidya, A.; Ravichandran, V.; Kashaw, S. K.; Agrawal, R. K. *Bioorg. Med. Chem.* **2012**, *20*, 3378.
20. Bailey, D. M.; Johnson, R. E.; Salvador, J. U. *J. Med. Chem.* **1973**, *16*, 1298.
21. Linington, R. G.; Williams, D. E.; Tahir, A.; Soest, R.; Andersen, R. J. *Org. Lett.* **2003**, *5*, 2735.
22. Srivastava, T.; Haq, W.; Katti, S. B. *Tetrahedron* **2002**, *58*, 7619.
23. Ramani, A. V.; Monika, A.; Indira, V. L.; Karyavardhi, G.; Venkatesh, J.; Jeankumar, V. U.; Manjashetty, T. H.; Yogeeswari, P.; Sriram, D. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2764.
24. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard, M7-A7*, 7th ed.; Wayne, PA, USA, 2006; p 26.
25. Pan, B.; Huang, R.; Han, S.; Qu, D.; Zhu, M.; Wei, P.; Ying, H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2461.