Antimicrobial activities of thiazole, imidazolidine, tetrahydropyrimidine derivatives and silver/polyvinyl alcohol nanocomposites against selected zoonotic fish bacterial pathogens

B. GHASEMI1, H. BEYZAEI2, S. H. HASHEMI3, M. GHAFFARI-MOGHADDAM2 AND M. MIRZAEI4

1Young Researchers and Elite Club, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran
2Department of Chemistry, Faculty of Science, University of Zabol, Zabol, Iran
3Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran
4Young Researchers and Elite Club, Quchan Branch, Islamic Azad University, Quchan, Iran
e-mail: hbeyzaei@yahoo.com

Bacterial infections appears to be one of the most important problems faced by the aquaculture sector. A wide range of antibiotics are being used to control the spread of aquatic pathogens (Santos and Ramos, 2016). Indiscriminate and irrational use of antibiotics has led to the emergence of drug resistant strains of several fish bacterial pathogens such as Streptococcus iniae, Edwardsiella tarda and Aeromonas hydrophila (Vivekanandhan et al., 2002; Park et al., 2009; Wei et al., 2011). These bacterial pathogens cause disease outbreak and mortality in farmed fishes which adversely affects profitability of aquaculture as well as public health (Vivekanandhan et al., 2002; Park et al., 2009; Wei et al., 2011). These three bacterial pathogens have been reported to be zoonotic and can cause diarrhea, gastroenteritis and skin lesions in human. The spread of antibiotic-resistant bacterial strains persuaded researchers to identify and synthesise new efficient antimicrobial agents such as metal nanoparticles and heterocyclic derivatives (Santos and Ramos, 2016).

Thiazole derivatives have been applied in the treatment of cancer, blood pressure and AIDS in human beings (Bakavoli et al., 2011). Antioxidant and anti-inflammatory effects of thiazoles have already been proven (Helul et al., 2013; Jaishree et al., 2013). They can be used to kill anopheles mosquitoes (Venugopla et al., 2013) and their inhibitory effects on Trypanosoma brucei and Candida spp. have been proved (Chementi et al., 2011; Zelisko et al., 2013). Furthermore, these compounds can inhibit the growth of a variety of Gram-positive and Gram-negative bacteria including Escherichia coli, Staphylococcus epidermidis, Enterococcus faecalis, Staphylococcus aureus, Streptococcus pyogenes and Pseudomonas fluorescens (Khalil et al., 2009; Bondock et al., 2010).

Imidazolidine derivatives have been shown to inhibit tumor cells; protozoan parasites such as Leishmania mexicana and Leishmania infantum as well as fungi like Rhizopus oryzae and Chrysosporium tropicum (Robert et al., 2003; Brahmaya et al., 2013). Various researchers have also shown their antibacterial effects on bacterial pathogens, including E. faecalis, E. coli and S. aureus (Wittine et al., 2012).

Tetrahydropyrimidine derivatives were found able to inhibit the bacterium Mycobacterium tuberculosis as well as fungi Aspergillus niger and Candida albicans (Akhaja et al., 2012). Some of their derivatives are efficient in the
treatment of Alzheimer’s and infectious diseases (Messer et al., 2000; Elumalaia et al., 2013). In vitro antimicrobial activities of these compounds have been studied against Klebsiella pneumoniae, Pseudomonas aeruginosa (Hussein et al., 2012) and S. aureus (Wittle et al., 2012).

A variety of therapeutic properties have been observed using silver nanoparticles. They can prevent the growth of liver and lung cancer cell lines, genus Leishmania and Rift Valley fever virus (Ahmad et al., 2014; Borrego et al., 2016; Rajeshkumar et al., 2016). Their antimicrobial activities have been studied on many bacterial pathogens such as K. pneumoniae, Bacillus subtilis and Streptococcus spp. (Rajeshkumar et al., 2016). In similar research, silver and gold nanoparticles were synthesised in the range size of 5-20 nm and their antimicrobial activities evaluated against A. hydrophila, Aeromonas bestiarum, P. fluorescens and E. tarda (Velmurugan et al., 2014).

In this study, inhibitory effects of some recently synthesised thiazole, imidazolidine and tetrahydropyrimidine derivatives as well as Ag/PVA nanocomposites were evaluated against three important zoonotic fish bacterial pathogens viz., S. iniae, E. tarda and A. hydrophila (Bakavoli et al., 2009; Bakavoli et al., 2011; Mohmedi-Kartalie et al., 2014; Beyzaei et al., 2015).

Thiazole derivatives 6a-d were synthesised in a three-step process (Bakavoli et al., 2009). A mixture of dinitrile 1 (1 mmol) and cysteamine (2) (1 mmol) in ethanol (2 ml) was stirred at room temperature for 4h to give thiazolidine 3. This compound (1 mmol) was treated with NaSH (2 mmol) in water (1 ml) at 50°C for 22 h to afford thioamide 4. Finally, thiazoles 6a-d were prepared from reaction of compound 4 (1 mmol), α-bromocarbonyls 5a-d (1 mmol) and NaHCO₃ (1 mmol) at room temperature for 2-8 h (Scheme 1).

| Table 1. Details of the derivatives of heterocyclic compounds |
|-------------------------------------------------------------|
| Heterocyclic compound | Derivative | Details |
|-----------------------|------------|---------|
| Thiazole              | 6a         | Ethyl 2-[(E)-cyanothiazolidin-2-ylidene]methyl]thiazole-4-carboxylate |
|                       | 6b         | (E)-2-(5-Acetyl-4-methylthiazol-2-yl)-2-(thiazolidin-2-ylidene)acetonitrile |
|                       | 6c         | Ethyl 2-[(E)-cyanothiazolidin-2-ylidene]methyl]-4-methylthiazole-5 carboxylate |
|                       | 6d         | (2E)-2-(4,5-Dihydro-4-oxothiazol-2-yl)-2-(thiazolidin-2-ylidene)acetonitrile |
|                       | 8          | (E)-2-(Benzol[d]thiazol-2(3H)-ylidene)-2-cyanoethanethioamide |
| Imidazolidine         | 10a        | 2-(Octahydro-2H-benzol[d]imidazol-2-ylidene)malononitrile |
|                       | 10b        | 2-(4,4-Dimethylimidazol-2-ylidene)malononitrile |
|                       | 10c        | 2-(4-Methylimidazolidin-2-ylidene)malononitrile |
| Tetrahydropyrimidine  | 10d        | 2-(5-Hydroxytetrahydropyrimidin-2(1H)-ylidene)malononitrile |
|                       | 10e        | 2-(Tetrahydropyrimidin-2(1H)-ylidene)malononitrile |

Scheme 1. Total synthesis of thiazoles 6a-d

Thiazole derivative 8 was synthesised from thionation of benzothiazole 7 (1 mmol) by P₂S₅ (2 mmol) in absolute ethanol (2 ml) (Scheme 2) (Bakavoli et al., 2011).

Scheme 2. Total synthesis of benzothiazole derivative 8

Imidazolidine derivative 10a-c and tetrahydropyrimidines 10d-e were produced via reaction of dinitrile 1 (1 mmol) and diaminoalkanes 9a-e (1 mmol) in ethanol (1 ml) at room temperature for 25-30 min (Scheme 3) (Beyzaei et al., 2015).

Scheme 3. Total synthesis of imidazolidine and tetrahydropyrimidine derivatives 10a-e

Details of the heterocyclic derivatives are presented in Table 1. The chemical structure of all synthesised compounds was characterised by elemental analysis, single crystal X-ray diffraction, ¹H NMR, ¹³C NMR and IR spectrometry. Afterwars, these derivatives were
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Solutions of antibiotics viz., gentamicin and penicillin were also prepared (as positive controls) by dissolving in double distilled water with initial concentration of 256 µg ml⁻¹.

S. iniae (ATCC 29178), E. tarda (ATCC 15947) and A. hydrophila (ATCC 7966) were provided from the Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. All bacteria were cultured in Mueller-Hinton agar medium (Merck®, Germany) at 37°C for 24 h except S. iniae which was grown at 25°C. Bacterial suspensions, at a concentration of 0.5 McFarland units (1.5 × 10⁸ cfu ml⁻¹), were prepared by measuring OD in a spectrophotometer at a wave length of 625 nm (Venkatesan et al., 2014). All biological tests were repeated three times, and the results were reported as an average of the three independent experiments.

Minimum inhibitory concentration (MIC) was estimated in sterile 96-well plate according to broth microdilution method. Mueller-Hinton broth medium (90 µl) and 10 µl of bacterial suspension (5 × 10⁸ cfu ml⁻¹) was added to each well in a row. One hundred microlitre each of various solutions of thiazole derivatives and antibiotics were added to the wells, so that their final concentrations were in the range of 32-4096 and 0.063-128 µg ml⁻¹ respectively. As negative control, DMSO was added to a separate well containing culture medium and bacterial suspension. Finally, the results were recorded after incubation for 24 h at 37°C (S. iniae at 25°C). The lucidity and turbidity in each well indicated lack or existence of bacterial growth, respectively. The lowest concentration that didn’t show any turbidity, was reported as MIC (Khalil et al., 2009).

The inhibition zone diameters (IZD) was measured according to disk diffusion method. Ten microlitre of bacterial suspension (1.5 × 10⁹ cfu ml⁻¹) was spread uniformly over the surface of Mueller-Hinton agar plate (Merck®, Germany). Ten microlitre each of initial concentrations of all compounds were poured on blank sterile discs (6.4 mm dia, 1 mm thickness, Padtan, Iran) and were placed on surface of the plates inoculated with bacterial suspension. Subsequently the plates were incubated for 24 h at 37°C (S. iniae at 25°C). Finally, IZD values were measured with a caliper (Khalil et al., 2009).

Antimicrobial properties of three different classes of heterocyclic compounds and Ag/PVA nanocomposites were studied against three aquatic pathogenic bacteria. The results of MIC and IZD values are shown in Table 2. Inhibitory effects against all bacteria (S. iniae, E. tarda and A. hydrophila) were observed for Ag/PVA nanocomposites. The IZD and MIC values were recorded in the range of 9.4-12.7 mm and 256-1024 µg ml⁻¹ respectively. The results were more evident on Gram-positive bacterium S. Iniae. Swain et al. (2014) also found more inhibitory power of silver nanoparticles on S. aureus in comparison with E. tarda and A. hydrophila. This was predictable because the cell wall of Gram-negative bacteria limits penetration of nanoparticles (Lemire et al., 2013). Mechanism of action of silver nanoparticles has not been known exactly, but damage to the cell membrane proteins and DNA are important factors reported in studies (Lemire et al., 2013).

Thiazoles 6a-c, imidazolidine derivatives 10a-c and tetrahydropyrimidins 10d-e could not prevent the growth of bacterial strains tested during the present study. In a similar research, antibacterial properties of tetrahydropyrimidine derivatives were evaluated on E. coli, S. aureus, S. epidermidis, B. subtilis and Bacillus mycoides and the results showed no effect of some derivatives (Prachayasittikul et al., 2010). This indicates the lack of broad-spectrum activity of these heterocyclic compounds. The ability of some imidazole derivatives to inhibit bacteria like S. aureus, E. coli, Micrococcus luteus, and P. aeruginosa is probably due to the presence of chlorine (Cl) and nitro (NO₂) substituents in their structure (Jamal abdul-Nasser et al., 2010; Shahid et al., 2014).
Thiazole derivatives 6d and 8 were the only heterocyclic compounds which were found effective during the study but, of course they had no effect on A. hydrophila. The maximum inhibitory effects of these compounds were recorded for E. tarda with MIC of 32 µg ml\(^{-1}\) and found more significant in comparison with some equivalent thiazoles (Pandeya et al., 1999). The structural study of thiazole 6d shows that it includes 4-thiazolone ring with a wide variety of antimicrobial activities (Zaky and Yousef, 2011). Also, the derivative 8 contains a thioamide substituent. This functional group is present in prothionamide antimycobacterial drug (Bartels and Bartels, 1998). Thiazole ring itself is a biologically active component and recently synthesised 2, 4-disubstituted hydrazinyl-thiazoles have been introduced as antibacterial, antioxidant and anticancer agents (Ghanbari Pirbasti and Mahmoodi, 2016). The best result in antibiogram test was observed with penicillin antibiotic against S. iniae with MIC value of 0.5 µg ml\(^{-1}\).

Although the observed in vitro antibacterial activities of heterocyclic compounds 6d and 8 were less than that of the antibiotics penicillin and gentamycin, more effective antibacterial agents can easily be synthesised via change in the substituents and functional groups on the starting materials, as structural skeletons of derivatives are preserved. In addition, antimicrobial activities of various transition metal complexes containing thiazoles 6d and 8 can also be evaluated.

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