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Note

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

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ABSTRACT

One of the serious problems faced by health and food security is the spread of drug-resistant zoonotic aquatic bacterial pathogens. For this reason, identification and application of new antibacterial agents are required. In this study, inhibitory effects of thiazole, imidazolidine and tetrahydropyrimidine derivatives and silver/polyvinyl alcohol (Ag/PVA) nanocomposites were evaluated against three important zoonotic fish bacterial pathogens namely *Streptococcus iniae*, *Edwardsiella tarda* and *Aeromonas hydrophila*. Ag/PVA nanocomposite was found to inhibit growth of all tested bacteria, the highest activity was observed against *S. iniae* with minimum inhibitory concentration (MIC) of 256 µg ml^{-1} and inhibition zone diameter (IZD) of 10.2 mm. No inhibitory effects were observed with imidazolidines10a-c, tetrahydropyrimidines 10d-e and thiazoles 6a-c. Among heterocyclic compounds, only thiazole derivatives 6d and 8 had inhibitory effects on *S. iniae* and *E. tarda* with MIC values of 32-256 µg ml^{-1} but lacked antibacterial activity against *A. hydrophila*.

Keywords: Antimicrobial effects, Ag/PVA nanocomposites, Fish bacterial pathogens, Heterocyclic compounds, Zoonotic

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 lilke *Rhizopus oryzae* and *Chrysosporium tropicum* (Robert *et al*., 2003; Brahmayya *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).

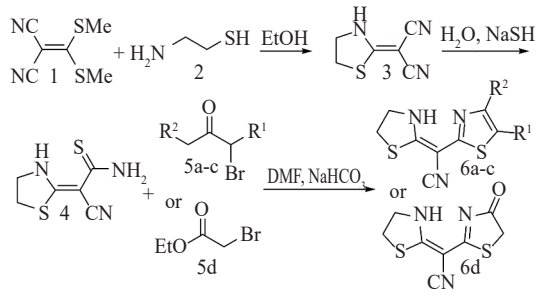
Tetrahydropyrimidine derivatives were found able to inhibit the bacterium *Mycobacterium tuberculosis* as well as fungi *Aspergillus niger* and *Candida albicans* (Akhaja *et al*., 2012). Some of their derivatives are efficient in the

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* (Wittine *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*., 2016; 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).

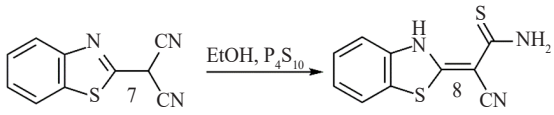
Thiazole derivatives 6a-d were synthesised in a three-step process (Bakavoli *et al*., 2009). A mixture of dinitrile 1 (1 mmol) and cysteamine (2) (1 mmol) in ethanol (2 ml) was stirred at room temprature for 4 h to give thiazolidine 3. This compound (1 mmol) was treated with NaSH (2 mmol) in water (1 ml) at 50°C for 22 h to afford thioamide 4. Finally, thiazoles 6a-d were prepared from reaction of compond 4 (1 mmol), *α*-bromocarbonyls 5a-d (1 mmol) and $NaHCO_3$ (1 mmol) at room temprature for 2-8 h (Scheme 1).



6a: R^1 = H, R^2 = CO_2Et; 6b: R^1 = $COCH_3$, R^2 = CH_3; 6c: R^1 = CO_2Et, R^2 = CH_3

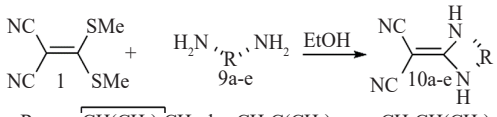
Scheme 1. Total synthesis of thiazoles 6a-d

Thiazole derivative 8 was synthesised from thionation of benzothiazole 7 (1 mmol) by P_2S_5 (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).



R = a: -$\overline{CH(CH_2)_4}$CH-, b: -$CH_2C(CH_3)_2$-, c: -$CH_2CH(CH_3)$-
d: -$CH_2CH(OH)CH_2$-, e: -$CH_2CH_2CH_2$-

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, ^{1}H NMR, ^{13}C NMR and IR spectrometry. Afterwards, these derivatives were

Table 1. Details of the derivatives of heterocyclic compounds

Heterocyclic compound	Derivative	Details
Thiazole	6a	Ethyl 2-[(*E*)-cyano(thiazolidin-2-ylidene)methyl]thiazole-4-carboxylate
	6b	(*E*)-2-(5-Acetyl-4-methylthiazol-2-yl)-2-(thiazolidin-2-ylidene)acetonitrile
	6c	Ethyl 2-((*E*)-cyano(thiazolidin-2-ylidene)methyl)-4-methylthiazole-5 carboxylate
	6d	(2*E*)-2-(4,5-Dihydro-4-oxothiazol-2-yl)-2-(thiazolidin-2-ylidene)acetonitrile
	8	(*E*)-2-(Benzo[*d*]thiazol-2(3*H*)-ylidene)-2-cyanoethanethioamide
Imidazolidine	10a	2-(Octahydro-2*H*-benzo[*d*]imidazol-2-ylidene)malononitrile
	10b	2-(4,4-Dimethylimidazolidin-2-ylidene)malononitrile
	10c	2-(4-Methylimidazolidin-2-ylidene)malononitrile
Tetrahydropyrimidine	10d	2-(5-Hydroxytetrahydropyrimidin-2(1*H*)-ylidene)malononitrile
	10e	2-(Tetrahydropyrimidin-2(1*H*)-ylidene)malononitrile

dissolved in dimethyl sulfoxide (DMSO) at an initial concentration of 8192 µg ml^{-1}.

The Ag/PVA nanocomposites were synthesised according to the procedure described by Mohmedi-Kartalie *et al.* (2014) as follows: Polyvinyl alcohol (0.4 g) was added to a solution comprising distilled water (20 ml) and acetic acid (0.3 ml). Subsequently, silver nitrate (0.001 g), benzoin (0.002 g) and *N*, *N*-dimethylformamide (5 ml) were added. The mixture was illuminated by a 1200 W UV lamp, as ultraviolet light source, for 1 min at 60-80˚C. Finally, the nanocomposites were oven-dried at 40˚C for 72 h. The FESEM image exhibited spherical shape of Ag nanoparticles in the average size of 20-30 nm (Fig. 1). A suspension of nanocomposites was prepared in double distilled water with initial concentration of 8192 µg ml^{-1}.

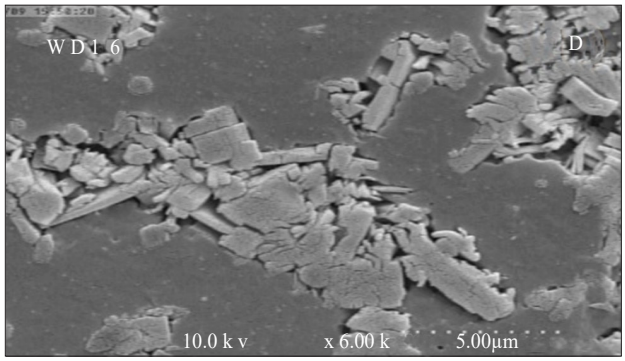


Fig. 1. FESEM image of Ag/PVA nanocomposite

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 (1.5×10^8 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 (5×10^5 cfu ml^{-1}) was added to each well in a row. One hundred microliter 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 difiusion method. Ten microlitre of bacterial suspension (1.5×10^8 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 (NO_2) substituents in their structure (Jamal abdul-Nasser *et al*., 2010; Shahid *et al*., 2014).

Table 2. The inhibition zone diameter (IZD, mm) and minimum inhibitory concentration (MIC, µg ml^{-1}) values of compounds

Compounds/Antibiotics	*S. iniae*		*E. tarda*		*A. hydrophila*	
	IZD	MIC	IZD	MIC	IZD	MIC
6a*	-	-	-	-	-	-
6b*	-	-	-	-	-	-
6c*	-	-	-	-	-	-
6d*	23.1	64	24.5	32	-	-
8*	14.3	256	22.5	32	-	-
10a*	-	-	-	-	-	-
10b*	-	-	-	-	-	-
10c*	-	-	-	-	-	-
10d*	-	-	-	-	-	-
10e*	-	-	-	-	-	-
Ag/PVA NC*	12.7	256	9.4	1024	10.3	512
Gentamicin**	16.3	4	17.1	8	19.1	2
Penicillin**	25.1	0.5	27.1	1	22.1	4

-: indicates no inhibitory effects at maximum concentration, *at initial concentration of 8192 µg ml^{-1}, **at initial concentration of 256 µg ml^{-1}

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 prothinoamide 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. İniae* 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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