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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Antimicrobial evaluation of 1,4-benzoxazine derivatives

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Abstract In this study, a series of *N*-unprotected 1,4-benzoxazine derivatives were evaluated for antibacterial activity. All the compounds showed low to good activity against Gram-positive and Gram-negative bacteria. Benzoxazine derivatives bearing nitro and trifluoromethyl groups are more potent than the other ones. Compounds with cyanide and ester groups have displayed poor activity. The compound **1c** has shown good activity against Gram-positive as well as Gram-negative bacteria.

Keywords Benzoxazine · Antibacterial · Disc diffusion method · Minimum inhibitory concentration · In vitro

Introduction

The emergence and spread of resistance in bacteria are complicating the treatment of serious infectious diseases and threatening to create species resistant to all currently available antimicrobial agents (Service, 1995; Walsh, 2000). Inappropriate and extensive use of antimicrobials provides a fertile environment for the resistant microorganisms to emerge, spread and persist. The alarming rates of microbial threats are major concerns to the global public health and scientific communities. In the European Union, Norway and Iceland, for example, 400,000 resistant infections are estimated to occur every year, leading to about 25,000 deaths, according to the European Centre for Disease Prevention and Control. Consequently, the discovery and development of new and different antimicrobial drugs having less susceptibility to the emergence of resistance are very important objective.

1,4-benzoxazine ring is endowed with various biological activities such as central nervous system depressants (Yang *et al*., 2004), antipsychotic agents (Birch *et al*., 1999), antagonists (Bohme *et al*., 2002) and antibacterial agents (Hayakawa *et al*., 1984; Li *et al*., 2010; Geng *et al*., 2011; Filippou *et al*., 2011; Xin *et al*., 2011), while others are potential drugs for treating neurodegenerative (Largeron *et al*., 1999), cardiovascular (Yang *et al*., 2004) and diabetic disorders (Rybczynski *et al*., 2004). Ofloxacin, one of the antimicrobial agents, possessing 1,4-benzoxazine core, was first patented in 1982 and received approval from the U.S. Food and Drug Administration (FDA) in 1990 (Fig. 1). The synthesis of 1,4-benzoxazine derivatives has relied predominantly upon multistep procedures, using 2-nitrophenols or 2-aminophenols as the starting materials (Ilaŝ *et al*., 2005; Achari *et al*., 2004; Ramesh *et al*., 2011). Diels–Alder reaction, even though old, is an attractive strategy for the synthesis of the 1,4-benzoxazine scaffold in one pot (Xu *et al*., 2006; Wolfer *et al*., 2006; Nicolaou *et al*., 2002; Heine *et al*., 1995). Recently, we have developed a novel methodology for the synthesis of variety

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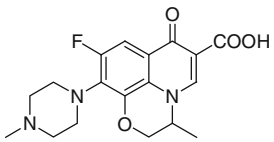
Fig. 1 Ofloxacin



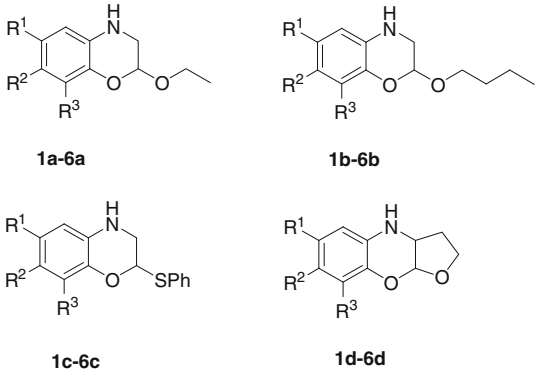


Fig. 2 Benzoxazine derivatives

of *N*-unprotected 1,4-benzoxazine derivatives using *o*-quinone monoimines and vinyl ethers via hetero inverse electron demand Diels–Alder reaction (Bodipati and Peddinti, 2012). From the literature, it is revealed that the benzoxazine derivatives having free *NH* group are more potent than the *N*-alkylated ones (Alper-Hayata *et al*., 2006). Moreover, benzoxazines bearing electron-withdrawing groups are showed more activity than the ones with electron-donating groups (Alper-Hayata *et al*., 2006). These observations prompted us to investigate the antimicrobial activity of differently substituted *N*-unprotected 1,4-benzoxazine derivatives (Fig. 2).

Results and discussions

The strains used in this study are Gram-positive bacteria, *Staphylococcus aureus* Microbial Type Culture Collection (MTCC), and Gram-negative bacteria, *Escherichia coli* MTCC. The antibacterial activity of 1,4-benzoxazine derivatives in comparison with that of control drugs, ofloxacin and tetracycline was determined by Kirby–Bauer disc diffusion method on Luria broth agar medium. The results of the antibacterial activity of the compounds are summarized in Table 1. Among the 24 compounds tested, 12 compounds displayed good in vitro antibacterial activity.

Compounds **4a**, **5a**, **4b**, **1c**, **4c**, **6c** and **5d** exhibited good activity and have shown zone of inhibition comparable to that of the control drugs, ofloxacin and tetracycline, against both *E. coli* and *S. aureus* (Table 1). Compounds **6a**, **5b**, **6b** and **5c** showed good activity against *E. coli* and moderate activity against *S. aureus.* On the other hand, benzoxazines **4d** and **6d** are more potent towards *S. aureus* than *E. coli*. From the results, it is clear that the

Table 1 The in vitro antimicrobial activity of benzoxazine derivatives and control drugs using Kirby–Bauer disc diffusion method

S. no.	Compound				Concentration (μg)	Zone of inhibition (mm)	
		R^1	R^2	R^3		*E. coli*	*S. aureus*
1	**1a**	CN	H	H	15	16	13
					20	17	14
2	**2a**	CN	H	OMe	15	14	10
					20	15	10
3	**3a**	COOMe	H	H	15	13	>8
					20	15	>8
4	**4a**	CF_3	H	H	15	20	19
					20	21	19
5	**5a**	NO_2	H	H	15	19	21
					20	20	21
6	**6a**	H	NO_2	H	15	21	17
					20	22	18
7	**1b**	CN	H	H	15	14	13
					20	14	14
8	**2b**	CN	H	OMe	15	12	15
					20	13	16
9	**3b**	COOMe	H	H	15	9	–
					20	10	–

Table 1 continued

S. no.	Compound				Concentration (μg)	Zone of inhibition (mm)	
		R^1	R^2	R^3		*E. coli*	*S. aureus*
10	**4b**	CF_3	H	H	15	18	18
					20	18	19
11	**5b**	NO_2	H	H	15	20	14
					20	20	15
12	**6b**	H	NO_2	H	15	19	14
					20	19	14
13	**1c**	CN	H	H	15	18	22
					20	19	23
14	**2c**	CN	H	OMe	15	12	14
					20	13	15
15	**3c**	COOMe	H	H	15	10	11
					20	10	12
16	**4c**	CF_3	H	H	15	19	20
					20	20	20
17	**5c**	NO_2	H	H	15	24	16
					20	24	16
18	**6c**	H	NO_2	H	15	22	18
					20	23	19
19	**1d**	CN	H	H	15	11	12
					20	12	13
20	**2d**	CN	H	OMe	15	9	11
					20	10	12
21	**3d**	COOMe	H	H	15	8	11
					20	9	11
22	**4d**	CF_3	H	H	15	14	17
					20	15	17
23	**5d**	NO_2	H	H	15	18	18
					20	19	19
24	**6d**	H	NO_2	H	15	15	17
					20	15	17
25	Ofloxacin[a]				5	21	18
26	Tetracyclin[a]				10	18	23

[a] Antibacterial activity of benzoxazine derivatives was compared with the standard drugs ofloxacin (5 μg) and tetracycline (10 μg)

benzoxazine derivatives carrying electron-withdrawing groups, such as nitro group in sixth and seventh positions and trifluoromethyl group in position six are more potent towards both bacteria. However, benzoxazine derivatives bearing cyanide and ester groups in position six have shown poor activity against both bacteria. Altering the substituent at 2-position of benzoxazine ring from ethoxy to butoxy did not improve the activity against both bacteria. Furthermore, the replacement of ethoxy group by –SPh group increased the antibacterial activity of the compound **1c** against both bacteria, and attachment of hydrofuran ring to the benzoxazine ring did not help improve the activity except compound **5d**.

The minimum inhibitory concentrations (MIC) were determined by broth dilution method using Luria broth. The strains were inoculated by inoculating device into LB medium, and the density of the cells was monitored by taking optical density (OD). The MIC values of the compounds are shown in Table 2.

Conclusion

In summary, we have evaluated in vitro the antibacterial activity of variety of *N*-unprotected 1,4-benzoxazine derivatives. Benzoxazine derivatives with electron-

Table 2 Minimum inhibitory concentration (μg) of benzoxazine derivatives

Compound	*E. coli*	*S. aureus*
4a	12.5	12.5
5a	25	12.5
6a	50	50
4b	25	25
5b	50	>100
6b	25	>100
1c	25	6.25
4c	12.5	12.5
5c	6.25	50
6c	12.5	50
5d	100	100

withdrawing groups, such as nitro and trifluoromethyl, have shown good activity against *E. coli* and *S. aureus* than the others. In particular, compounds with –SPh group in position-2 of the benzoxazine ring showed more activity towards both bacteria.

Antimicrobial studies

The in vitro antimicrobial studies were carried out by Kirby–Bauer disc diffusion method against *E. coli* and *S. aureus*. The growth of the bacteria was monitored by taking OD. When the OD reaches to 0.4–0.6 at 600 nm, then the medium was centrifuged to obtain pellet. Luria broth agar plates were swabbed with the pellet, and the discs of Whatman filter paper having diameter of 5 mm were incorporated into the plates equally. The compounds were dissolved in DMSO of 5 mg/ml, and from this, 3 and 4 μl were added into the discs. Simultaneously, the standard antibiotics, ofloxacin and tetracycline, were tested against same bacteria (as positive controls). The compounds were dissolved in DMSO which showed no zone of inhibition acts as negative control. The plates were incubated at 37 °C for 24 h after which the zone of inhibition of disc was measured. Duplicates were maintained, and the average values were calculated eventual antibacterial activity.

Broth dilution method was used to determine MIC of the above-mentioned compounds. Freshly prepared Luria broth was used as diluents. The culture of the bacteria having OD 0.4–0.6 is diluted 100-folds in Luria broth (100 μl bacterial cultures in 10 ml of LB). The stock solution of the compounds was prepared by dissolving 3.125 mg in 1 ml of DMSO. The solution of the compounds was added in increasing order (1, 2, 4, 8, 16 and 32 μl of stock solution contains 3.125, 6.25, 12.5, 25, 50 and 100 μg of the compounds) to the test tubes containing bacterial culture. The test tubes were incubated at 37 °C for 24 h. The tubes were examined for visible growth of bacteria with LB as control. The lowest concentration which inhibited the visible growth of bacteria was taken as MIC.

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