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Synthesis of new benzisoxazole derivatives and their antimicrobial, antioxidant and anti-inflammatory activities

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ABSTRACT

A series of benzisoxazole derivatives were synthesized and evaluated for their antibacterial, antioxidant and anti-inflammatory activities. The results indicated that most of the compounds exhibit moderate antimicrobial activity against Gram negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Shigella flexineri*) and Gram positive (*Bacillus subtilis*) bacterial culture. The molecules were evaluated for antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl scavenging, super oxide radical scavenging and hydroxyl radical scavenging assays and most of them showed good antioxidant activities. Also, the synthesized compounds were screened for anti-inflammatory activities such as lipoxygenase inhibition and indirect haemolytic assays.

1. Introduction

Benzisoxazole derivatives are one of the most important heterocyclic system in the field of medicinal chemistry and associated with the wide range of biological activities such as antimicrobial [1], anticonvulsant [2,3], antitumor [4,5], anti-psychotic [6-8], antithrombotic [9] analgesic activities [10]. They have also exhibited antiglycating [11] and cholinesterase-inhibiting properties [12-13]. Previously we have investigated various biological activities of these benzisoxazole derivatives as antimicrobial [1] and cholinesterase-inhibiting agents [13]. Recently, we have also reported some derivatives of piperidine conjugated benzisoxazole derivatives as antibacterial, anti-oxidant and anti-inflammatory agents [14]. In continuation of these works, we extended our efforts towards the synthesis of new benzisoxazolylpiperidinesulfonyl derivatives and the study of their antibacterial, antioxidant and anti-inflammatory activities.

2. Experimental

2.1. Instrumentation

The melting points were determined on Selaco melting point apparatus and are uncorrected. Infrared spectra were recorded on Shimadzu FT-IR model 8300 spectrophotometer. ^{1}H NMR spectra were recorded on an NMR spectrometer operating at 400MHz using TMS as internal standard. Mass spectra were recorded using electrospray ionization mass spectrometry. The C, H and N analysis were performed using CE-400 CHN analyzer. Reactions were monitored by TLC using pre-coated sheets of silica gel G/UV-254 of 0.25 mm thickness (Merck 60F_{254}) using UV light for visualization. All chemicals were obtained from Aldrich, Fluka and Merck Chemicals.

2.2. Synthesis

8-*Tert*-butyl 4-methyl 3-methyl-2-oxo-1-oxa-8-azaspiro [4.5]dec-3-ene-4,8-dicarboxylate (**3**), 8-(*tert*-butoxycarbonyl)-3-methyl-2-oxo-1-oxa-8-azaspiro[4.5]dec-3-ene-4-carboxylic acid (**4**), *tert*-butyl 4-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-3-methyl-2-oxo-1-oxa-8-azaspiro[4.5]dec-3-ene-8-carboxylate (**6**), 4-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidine-1-carbonyl)-3-methyl-1-oxa-8-azaspiro[4.5]dec-3-en-2-one hydrochloride (**7**) are synthesized according to reference [14].

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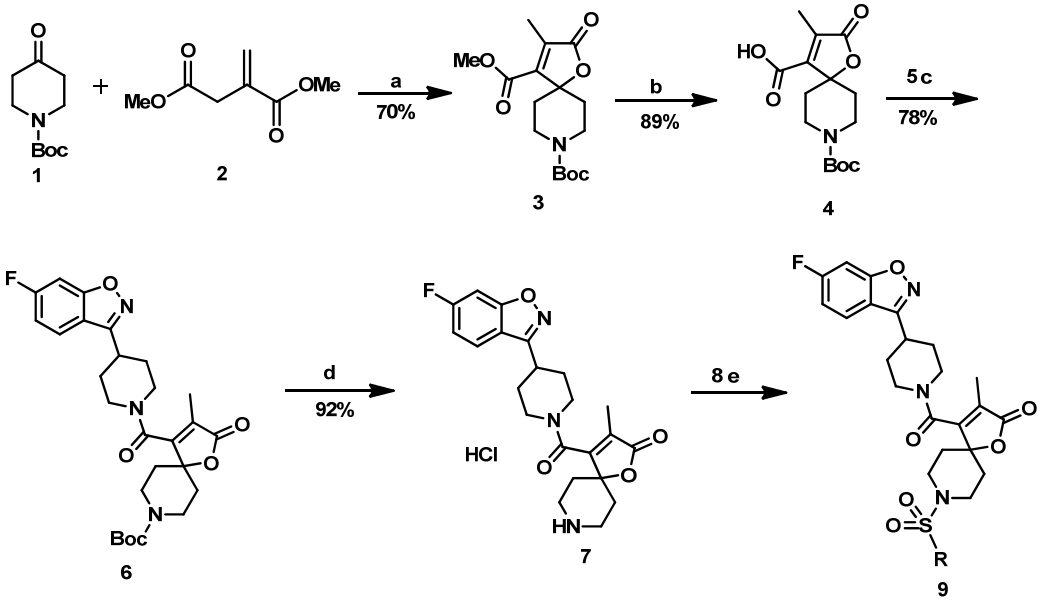
Table 1. Derivatives of benzisoxazole.

Entry	R (8, 9)	Compound	Yield (%)
1	4-MeC_6H_4	9a	76
2	2,5-$Cl_2C_6H_3$	9b	71
3	4-$MeOC_6H_4$	9c	75
4	C_6H_5	9d	80
5	4-FC_6H_4	9e	65
6	2-$NO_2C_6H_4$	9f	74
7	4-$NO_2C_6H_4$	9g	68
8	4-ClC_6H_4	9h	79



Reagents and reaction conditions: (a) MeONa/THF, 0 °C-RT, 8 h. (b) LiOH/MeOH/H_2O, 0 °C-RT, 3 h. (c) 6-fluoro-3-(piperidin-4-yl)benzo[d]isoxazole hydrochloride **5**, EDC.HCl/HOBt/DIPEA/CH_2Cl_2, 0 °C-RT, 8h. (d) HCl/ether, 0 °C-RT, 1h. (e) RSO_2Cl **8**, TEA/EDC, 0 °C-RT, 3-4 h.

Scheme 1

2.2.1. General procedure for the synthesis of compounds 9a-h

To a solution of compound **7** (5 mmol) and triethyl amine (5 mmol) in dichloromethane (20 mL); sulfonyl chloride **8** (5 mmol) was added at 0 °C and stirred at room temperature for 3-4 h. After the completion of the reaction, 20 mL of water was added and extracted the reaction mixture with dichloromethane (20 mL × 2). The organic layer was concentrated under reduced pressure to get products **9** (Scheme 1, Table 1) which were purified by column chromatography using $CHCl_3$:MeOH (9:1, *v:v*) as eluent.

4-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-3-methyl-8-tosyl-1-oxa-8-azaspiro[4.5]dec-3-en-2-one (**9a**): Colour: White. Yield: 76%. M.p.: 104-106 °C. FT-IR (KBr, ν, cm^{-1}): 1660 (Amide CO str.), 1740 (Ester CO str.), 3045 (Ar. CH str.). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.09 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.78 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.64 (d, 1H, *J* = 7.2 Hz, Ar-H), 7.26 (d, 1H, *J* = 7.2 Hz, Ar-H), 7.05 (s, 1H, Ar-H), 3.30-3.39 (m, 8H, CH_2), 2.75 (m, 1H, CH), 2.54 (s, 3H, CH_3), 2.35 (S, 3H, Ar-Me), 1.65-1.90 (m, 8H, CH_2). MS (ESI, *m/z*): 568 (M+1). Anal. calcd. for $C_{29}H_{30}FN_3O_6S$: C, 61.36; H, 5.33; N, 7.40. Found: C, 61.41; H, 5.39; N, 7.47%.

8-((2,5-Dichlorophenyl)sulfonyl)-4-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-3-methyl-1-oxa-8-azaspiro[4.5]dec-3-en-2-one (**9b**): Colour: White. Yield: 71%. M.p.: 110-112 °C. FT-IR (KBr, ν, cm^{-1}): 1655 (Amide CO str.), 1756 (Ester CO str.), 3049 (Ar. CH str.). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.77 (s, 1H, Ar-H), 7.469-7.55 (m, 3H, Ar-H), 7.28 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.03 (s, 1H, Ar-H), 3.25-3.35 (m, 8H, CH_2), 2.72 (m, 1H, CH), 2.50 (s, 3H, CH_3), 1.62-1.88 (m, 8H, CH_2). MS (ESI, *m/z*): 623 (M+1). Anal. calcd. for $C_{28}H_{26}Cl_2FN_3O_6S$: C, 54.02; H, 4.21; N, 6.75. Found: C, 54.09; H, 4.26; N, 6.81%.

4-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-8-((4-methoxyphenyl)sulfonyl)-3-methyl-1-oxa-8-azaspiro[4.5]dec-3-en-2-one (**9c**): Colour: White. Yield: 75% (2.18 g). M.p.: 98-100 °C. FT-IR (KBr, ν, cm^{-1}): 1659 (Amide CO str.), 1782 (Ester CO str.), 3047 (Ar. CH str.). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.91 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.56 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.15-7.24 (m, 3H, Ar-H), 6.97 (s, 1H, Ar-H), 3.82 (s, 3H, OMe), 3.32-3.45 (m, 8H, CH_2), 2.66 (m, 1H, CH), 2.52 (s, 3H, CH_3), 1.62-1.85 (m, 8H, CH_2). MS (ESI, *m/z*): 584 (M+1). Anal. calcd. for $C_{29}H_{30}FN_3O_7S$: C, 59.68; H, 5.18; N, 7.20. Found: C, 59.75; H, 5.26; N, 7.29%.

4-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-3-methyl-8-(phenylsulfonyl)-1-oxa-8-azaspiro[4.5]dec-3-en-2-one (**9d**): Colour: White. Yield: 80%. M.p.: 120-122 °C. FT-IR (KBr, ν, cm^{-1}): 1659 (Amide CO str.), 1765 (Ester CO str.), 3055 (Ar. CH str.). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.26 (s, 1H, Ar-H), 8.04 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.95 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.58 (m, 2H, Ar-H), 7.28 (m, 2H, Ar-H), 7.05 (s, 1H, Ar-H), 3.25-3.35 (m, 8H, CH_2), 2.70 (m, 1H, CH), 2.52 (s, 3H, CH_3), 1.60-1.84 (m, 8H, CH_2). MS (ESI, *m/z*): 554 (M+1). Anal. calcd. for $C_{28}H_{28}FN_3O_6S$: C, 60.75; H, 5.10; N, 7.59. Found: C, 60.82; H, 5.18; N, 7.65%.

4-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-8-((4-fluorophenyl)sulfonyl)-3-methyl-1-oxa-8-azaspiro[4.5]dec-3-en-2-one (**9e**): Colour: White. Yield: 65%. M.p.: 110-112 °C. FT-IR (KBr, ν, cm^{-1}): 1665 (Amide CO str.), 1755 (Ester CO str.), 3068 (Ar. CH str.).

Table 2. Antibacterial activity of benzisoxazoles **9a-h**.

Compound	Zone of inhibition in millimetre *				
	Escherichia coli	*Bacillus subtilis*	*Klebsiella pneumoniae*	*Salmonella typhi*	*Shigella flexneri*
9a	11	10	11	10	-
9b	12	-	14	12	-
9c	12	10	12	12	-
9d	13	10	22	19	13
9e	11	11	16	18	-
9f	11	13	9	14	10
9g	11	-	10	10	-
9h	12	10	12	11	-

* Inhibition zones including disc (6 mm) diameter, Positive control zone is 35 to 40 mm, "-"= Not active.

Table 3 Antioxidant activity of benzisoxazoles **9a-h**.

Compounds	IC_{50} values in µM		
	DPPH radical Scavenging Assay	Hydroxyl radical Scavenging assay	Superoxide radical scavenging assay
9a	3.8	3.7	2.3
9b	7.4	7.6	5.0
9c	4.0	4.1	2.5
9d	6.8	7.0	6.9
9e	8.8	7.8	9.4
9f	5.4	5.7	7.0
9g	5.2	4.7	6.6
9h	7.0	6.6	4.3
Ascorbic acid	3.5	3.4	2.8

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.24 (s, 1H, Ar-H), 8.04 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.97 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.54 (m, 2H, Ar-H), 7.24 (m, 1H, Ar-H), 7.02 (s, 1H, Ar-H), 3.25-3.36 (m, 8H, CH_2), 2.71 (m, 1H, CH), 2.52 (s, 3H, CH_3), 1.61-1.83 (m, 8H, CH_2). MS (ESI, *m/z*): 572 (M+1). Anal. calcd. for $C_{28}H_{27}F_2N_3O_6S$: C, 58.84; H, 4.76; N, 7.35. Found: C, 58.89; H, 4.81; N, 7.41%.

4-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-3-methyl-8-((2-nitrophenyl)sulfonyl)-1-oxa-8-azaspiro[4.5]dec-3-en-2-one (**9f**): Colour: White. Yield: 74%. M.p.: 134-136 °C. FT-IR (KBr, ν, cm^{-1}): 1665 (Amide CO str.), 1771 (Ester CO str.), 3032 (Ar. CH str.). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 9.02 (d, 2H, *J* = 2.8 Hz, Ar-H), 8.94 (m, 2H, Ar-H), 7.56 (d, 1H, *J* = 7.2 Hz, Ar-H), 7.33 (d, 1H, *J* = 7.2 Hz, Ar-H), 7.02 (s, 1H, Ar-H), 3.26-3.39 (m, 8H, CH_2), 2.72 (m, 1H, CH), 2.49 (s, 3H, CH_3), 1.66-1.81 (m, 8H, CH_2). MS (ESI, *m/z*): 599 (M+1). Anal. calcd. for $C_{28}H_{27}FN_4O_8S$: C, 56.18; H, 4.55; N, 9.36. Found: C, 56.26; H, 4.62; N, 9.42%.

4-(4-(6-Fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-3-methyl-8-((4-nitrophenyl)sulfonyl)-1-oxa-8-azaspiro[4.5]dec-3-en-2-one (**9g**): Colour: White. Yield: 68%. M.p.: 140-142 °C. FT-IR (KBr, ν, cm^{-1}): 1669 (Amide CO str.), 1771 (Ester CO str.), 3061 (Ar. CH str.). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.71 (s, 1H, Ar-H), 8.53 (d, 1H, *J* = 8.0 Hz, Ar-H), 8.43 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.89 (t, 1H, *J* = 7.8 Hz, Ar-H), 7.56 (d, 1H, *J* = 7.2 Hz, Ar-H), 7.26 (d, 1H, *J* = 7.3 Hz, Ar-H), 6.96 (s, 1H, Ar-H), 3.27-3.42 (m, 8H, CH_2), 2.67 (m, 1H, CH), 2.47 (s, 3H, CH_3), 1.68-1.82 (m, 8H, CH_2). MS (ESI, *m/z*): 599 (M+1). Anal. calcd. for $C_{29}H_{27}FN_4O_8$: C, 56.18; H, 4.55; N, 9.36. Found: C, 56.28; H, 4.64; N, 9.45%.

8-((4-Chlorophenyl)sulfonyl)-4-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperidine-1-carbonyl)-3-methyl-1-oxa-8-azaspiro[4.5]dec-3-en-2-one (**9h**): Colour: White. Yield: 79%. M.p.: 128-130 °C. FT-IR (KBr, ν, cm^{-1}): 1667 (Amide CO str.), 1774 (Ester CO str.), 3059 (Ar. CH str.). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.53-7.58 (m, 2H, Ar-H), 7.19-7.25 (m, 4H, Ar-H), 6.95 (s, 1H, Ar-H), 3.31-3.45 (m, 8H, CH_2), 2.71 (m, 1H, CH), 2.53 (s, 3H, CH_3), 1.62-1.85 (m, 8H, CH_2). MS (ESI, *m/z*): 588 (M+1). Anal. calcd. for $C_{28}H_{27}ClFN_3O_6S$: C, 57.19; H, 4.63; N, 7.15. Found: C, 57.26; H, 4.69; N, 7.21%.

2.3. Biological evaluation-antibacterial, antioxidant and anti-inflammatory activities

2.3.1. Antibacterial activity

Antibacterial tests were carried out by disc diffusion method using 100 µL of suspension containing 10^6 cells/mL of bacteria. The discs (6 mm diameter) were impregnated with 5 mg and 10 mg/mL of each compound and placed on the inoculated nutrient agar. Then, the inoculated plates were incubated at 37±0.1 °C at 24 h. One antibacterial drug, chloramphenicol was used as positive control. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms and the results are summarised in Table 2.

2.3.2. Antioxidant activity

2.3.2.1. DPPH radical scavenging assay

DPPH radical scavenging assays [15] were performed in 300 µL reaction mixtures containing 200 µL of 0.1 mM DPPH–ethanol solution, 90 µL of 50 mM Tris-HCl buffer (pH = 7.4), and 10 µL of deionised water (as control) and various concentrations of compounds **9a-h** (1.7-8.8 µg/mL). Ascorbic acid was used as a standard. After 30 min of incubation at room temperature, absorbance (540 nm) of the reaction mixtures was taken by a plate reader (Lab systems Mullikan MS). The percentage radical scavenging activity was calculated according to the following formula:

Inhibition (%) = (Absorbance control-Absorbance Sample/ Absorbance Control) × 100 (1)

The DPPH radical scavenging activity is demonstrated in Figure 1 and Table 3.

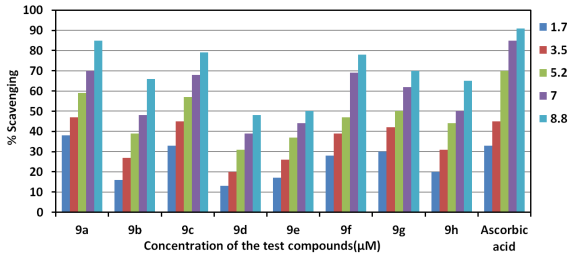


Figure 1. DPPH radical scavenging assay.

Table 4. Anti-inflammatory activity of benzisoxazoles **9a-h** *.

Compounds	IC_{50} values in µM	
	Lipoxygenase inhibition assay	PLA_2 inhibition assay
9a	3.4	44.0
9b	7.8	68.4
9c	3.7	50.4
9d	9.5	76.6
9e	7.8	73.1
9f	4.0	46.4
9g	3.7	35.0
9h	6.5	56.3
Indomethacin	3.5	-
Aristolochic acid	-	30.0

* "-"= Not determined.

2.3.2.2. Hydroxyl radical scavenging assay

The reaction mixture in final volume of 2 mL containing 0.1 mL of EDTA (1 mM), 0.01 mL of $FeCl_3$ (10 mM), 0.1 mL of H_2O_2 (10 mM), 0.36 mL of deoxyribose (10 mM), 1 mL of the compounds **9a-h** (concentrations from 1.7-8.8 µg/mL), 0.33 mL of phosphate buffer (50 mM, pH = 7.4) and 0.1 mL ascorbic acid (1 mM) added in sequence. The mixture was incubated at 37 °C for 1 h. 1 mL of the incubated mixture was mixed with 1 mL of 10% trichloro acetic acid and 1 mL of TBA (1% in 0.025 M NaOH), the resulting mixture was incubated in water bath at 90 °C for 20 min to develop a pink chromogen which was measured at 532 nm [16]. Ascorbic acid was used as a positive control. Percentage inhibition was evaluated by using the equation

Inhibition (%) = (Absorbance control-Absorbance Sample/ Absorbance Control) × 100 (2)

The potency of benzisoxazoles for hydroxyl radical scavenging activity is illustrated in Figure 2 and Table 3.

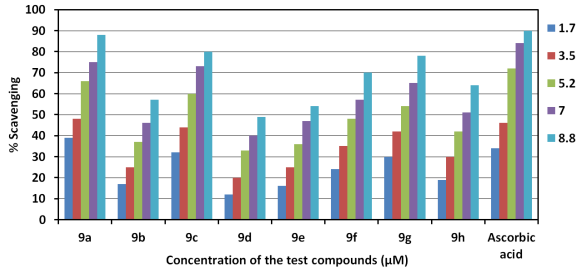


Figure 2. Hydroxyl radical scavenging assay.

2.3.2.3. Superoxide anion radical scavenging assay

The percentage inhibition of superoxide radical at 1 mL of NBT (156 µM NBT in 100 mM phosphate buffer of pH = 7.4), 1 mL of NADH (468 µM in 100 mM phosphate buffer of pH = 7.4) and varying concentration of compounds **9a-h** (1.1-5.8 µg/mL) were mixed to give a final volume of 3 mL. The reaction was started by the addition of 100 µL of PMS (60 µM in 100 mM phosphate buffer of pH = 7.4). The reaction mixture was incubated at 25 °C for 5 min and the absorbance was measured at 560 nm. Ascorbic acid was used as a standard [17]. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity and it is illustrated in Figure 3 and Table 3.

2.3.3. Anti-inflammatory activity

2.3.3.1. Lipoxygenase inhibition assay

Lipoxygenase inhibition assay [18] was carried out using linoleic acid as substrate and lipoxgenase enzyme. To a solution of 0.1 mL of 2 M borate buffer (pH = 9.0), 0.1 mL of 1000 units lipoxidase enzyme, solution of compounds **9a-h** in DMSO (1 mg/mL) was added and incubated with the enzyme with various concentrations. The tubes were agitated and incubated at room temperature for 5 min, after which 2.0 mL of substrate solution, 0.6 mM linoleum acid were added, mixed well and the absorbance was measured spectrophotometrically for 4 min at 234 nm (Shimadzu-2401 PC). Indomethacin was used as a reference standard drug. Percentage (%) inhibition was calculated by the following equation

Inhibition (%) = (Absorbance control-Absorbance Sample/ Absorbance Control) × 100 (3)

The lipoxygenase inhibition activity of benzisoxazole is summarised in Table 4 and Figure 4.

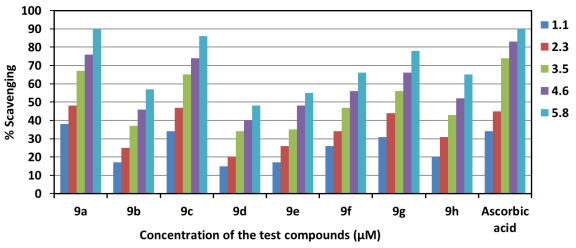


Figure 3. Superoxide radical scavenging assay.

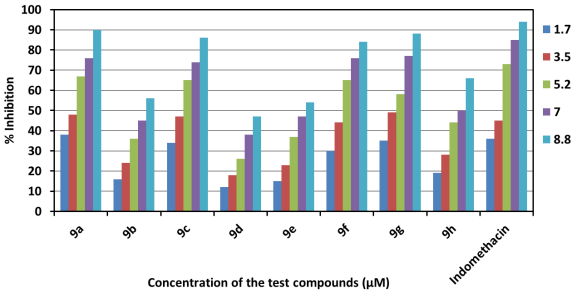


Figure 4. Lipoxygenase inhibition assay.

2.3.3.2. Inhibition of PLA_2 induced haemolysis in human erythrocytes

The substrate for indirect hemolytic activity was prepared by suspending 1 mL of fresh human red blood cells and 1 mL of fresh Hen's egg yolk in 8 mL of phosphate buffered saline. 1 mL of suspension was incubated with 4-28 µg of partially purified venom for 45 min at 37 °C and the reaction was stopped by the

addition of 9 mL of ice cold PBS. The suspension was centrifuged at 2000 rpm for 20 min and then the released haemoglobin was read at 540 nm. 10 µg of venom sample (secretory-PLA_2 purchased from sigma) was incubated with various concentration of compounds **9a**-**h** (1 mg/mL in DMSO) for 30 mins at room temperature and 1 mL of substrate was added, again incubated for 30 mins at room temperature and the reaction was stopped by adding 9 mL of ice cold PBS to all test tubes and centrifuged at 2000 rpm for 10 mins. Finally absorbance was measured at 540 nm [19] and inhibitory activities are summarised in Table 4 and Figure 5.

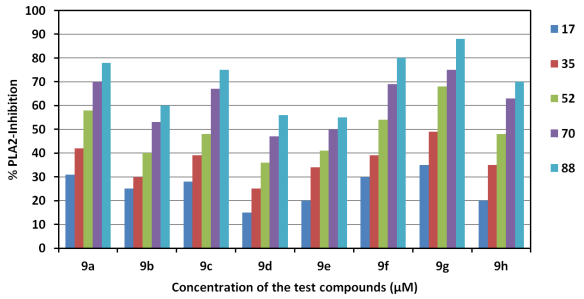


Figure 5. Inhibition of PLA2 induced haemolysis.

3. Results and discussion

The key intermediate 4-(4-(6-fluorobenzo[d]isoxazol-3-yl) piperidine-1-carbonyl)-3-methyl-1-oxa-8-azaspiro[4.5]dec-3-en-2-one hydrochloride **7** was prepared according to our earlier reported procedure [14]. Thus, cyclocondensation of *tert*-butyl 4-oxopiperidine-1-carboxylate, **1**, with dimethyl 2-methylenesuccinate, **2**, in presence of sodium methoxide in THF gave 8-*tert*-butyl 4-methyl 3-methyl-2-oxo-1-oxa-8-azaspiro[4.5]dec-3-ene-4,8-dicarboxylate, **3**, in 70% yield. Selective hydrolysis of methyl ester group in compound **3** by lithium hydroxide in methonolic water was carried out to get 8-(*tert*-butoxycarbonyl)-3-methyl-2-oxo-1-oxa-8-azaspiro[4.5] dec-3-ene-4-carboxylic acid, **4**, in 89% yield. Condensation of compound **4** with 6-fluoro-3-(piperidin-4-yl)benzo[d]isoxazole hydrochloride, **5**, [20] in presence of 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide hydrochloride (EDC.HCl) and hydroxyl benzotriazole (HOBt) in dichloromethane to afford *tert*-butyl 4-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperi-dine-1-carbonyl)-3-methyl-2-oxo-1-oxa-8-azaspiro[4,5]dec-3-ene-8-carboxylate, **6**. Cleavage of *tert*-butyl oxy group in compound **6** by saturated solution of hydrochloric acid in ether to give the key intermediate 4-(4-(6-fluorobenzo[d]isoxazol-3-yl)piperi-dine-1-carbonyl)-3-methyl-1-oxa-8-azaspiro[4.5]dec-3-en-2-one hydrochloride, **7**. Sulfonation of compound **7** was carried out with various sulfonyl chlorides **8** to get final products **9**.

All compounds showed moderate antibacterial activity against *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi* and *Bacillus subtilis*. While all compounds except **9d** and **9f** were inactive against *Shigella flexneri*. Compound **9d** without any substituent on phenyl ring showed highest antibacterial activity. In all the anti-oxidant assays compounds **9a** and **9c** containing methyl and methoxy substituent showed good inhibitory activity. The remaining compounds showed moderate anti-oxidant activity. However, it is not possible to give any rational explanation for the antioxidant activities of these compounds even in the absence of essential enolic group. In both lipoxygenase inhibition and PLA2 inhibition assays, compounds **9a** bearing methyl group at 4-position of phenyl ring and compounds **9f** and **9g** bearing nitro group at 2- and 4-position of phenyl ring respectively exhibited good anti-inflammatory activity, the remaining compounds showed moderate activity.

4. Conclusion

In summary, we have synthesized a new series of benzisoxazole derivatives in good yields and evaluated for their antibacterial, antioxidant and anti-inflammatory activities. Benzisoxazole derivative without substitution on phenyl ring showed good antibacterial activity against *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi* and *Bacillus subtilis*. Benzisoxazoles **9a** and **9c** bearing methyl and methoxy substituents exhibited prominent antioxidant activity and **9f** and **9g** bearing electron withdrawing nitro group showed good anti-inflammatory activity. Thus a new class of benzisoxazole derivatives can be incorporated to the family of bioactive heterocyclic compounds. It should also be noted that, in general compounds bearing activating groups on phenyl ring showed good antioxidant activities, and those with deactivating groups exhibited anti-inflammatory activities.

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