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Original Article

SYNTHESIS AND EVALUATION OF ANTI-INFLAMMATORY AND ANTIBACTERIAL ACTIVITIES OF SOME 1, 2-BENZISOXAZOLE DERIVATIVES

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ABSTRACT

Objective: A series of 1, 2-Benzisoxazole derivatives were synthesized and characterized by various analytical techniques like Melting point, Rf, FTIR, and NMR spectra.

Methods: Structures of the compounds were elucidated and evaluated for anti-inflammatory activity by HRBC membrane stabilization method, antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* by cup plate method.

Results: The compounds 4a and 4e showed good anti-inflammatory activity compared with standard drug Diclofenac sodium and compounds 4b and 4d showed good antibacterial activity on compared with standard drug Gentamycin.

Conclusion: These compounds may serve as future leads for anti-inflammatory and antibacterial drug discovery.

Keywords: 1, 2-Benzisoxazole, Anti-inflammatory, Antibacterial activity

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INTRODUCTION

The nucleus selected for the present work is 1, 2 benzisoxazole which is a versatile molecule. Benzisoxazole is an aromatic organic compound with a molecular formula C_7H_5NO containing a benzene fused isoxazole ring structure. Benzisoxazole has no household use. It is used primarily in industry and research. Being a heterocyclic compound, benzisoxazole finds use in research as a starting material for the synthesis of larger, usually bioactive structures. It is found within the chemical structures of pharmaceutical drugs such as the antipsychotic Risperidone [1] and the anticonvulsant Zonisamide [2-4]. Its aromaticity makes it relatively stable, although as a heterocycle, it has reactive sites which allow for functionalization. Molecules with substituted 1, 2-benzisoxazole often exhibit anti-inflammatory [5], analgesic, antifungal [6], antioxidant [5], analgesic [7], and antibacterial activity [8].

MATERIALS AND METHODS

All the chemicals and reagents used in the synthesis of titled compounds were of the analytical or synthetic grade. The melting points of synthesized compounds were determined by open capillary tube method, using liquid paraffin and are uncorrected. Infra-red spectra of the compounds were recorded using KBr pellets in the range of 4000-500 cm^{-1} on Jasco FTIR model 6200. ^1H NMR (300 MHz) spectra were recorded on Bruker Avance DPX 300 instruments using DMSO as solvent and Tetramethyl silane (TMS) as an internal standard; Chemical shifts were recorded in parts per million (PPM). Analytical thin-layer chromatography (TLC) was performed on precoated TLC sheets of silica gel using toluene: chloroform: methanol (9: 3: 1) as mobile phase; Iodine vapour was used to identify the location of spots.

Experimental section

In the synthetic scheme, different aromatic aldehydes were treated with O-hydroxy acetophenone in ethanol with a catalytic amount of 40% KOH, stirred and kept at RT for 24 hr, acidified with conc. HCl to form chalcones by Aldol condensation. It involves the acid-base reaction between a strong base (hydroxide ion) and a hydrogen located alpha to a carbonyl group and forms an enolate. The enolate attacks the carbonyl group of aromatic aldehyde without alpha hydrogens. The aldol undergoes an acid-base reaction with the remaining acidic α-hydrogen is lost, followed by the loss of OH-as a leaving group to give an enal. The net loss of H+and OH-represents the loss of water. Various chalcones were treated with Hydroxylamine hydrochloride and potassium carbonate under reflux in ethanol; nucleophilic attach by the basic nitrogen compound (Hydroxylamine) on carbonyl carbon (Nucleophilic addition) to form Benzisoxazole derivatives [8].

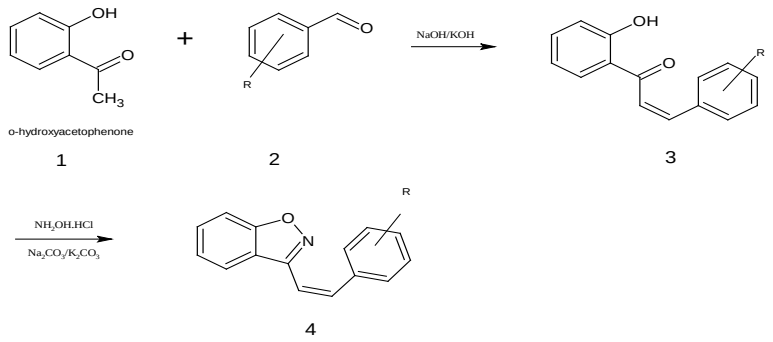


Fig. 1: Scheme for the synthesis of 1, 2-benzisoxazole derivatives

Synthesis of [3-[2-phenylethenyl]-1, 2-benzisoxazole] (4a)

Step-1: A mixture of o-hydroxy acetophenone (0.01 M) and Benzaldehyde (20% 0.04 M) was dissolved in 10 ml ethanol. To this mixture 10 ml of 40% KOH was added, the reaction mixture was stirred and kept at RT for 24 hr. Then the reaction mixture was poured over crushed ice and contents were acidified with concentrated hydrochloric acid. Chalcone thus obtained was purified by recrystallization from ethanol [8].

Step-2: A mixture of 2.77 gm of chalcone (10 mmol), 0.7 gm of Hydroxylamine hydrochloride (10 mmol) and 1.4 gm of anhydrous potassium carbonate (10 mmol) in 50 ml of ethanol was refluxed for 8 h, then left to cool. The reaction mixture was poured into cold water, and the solid product was filtered off, washed with water, dried and finally crystallized from ethanol to afford Benzisoxazole derivative [8].

Synthesis of [3-[2-(2-chlorophenyl) ethenyl]-1,2-benzisoxazole] (4b)

Step-1: A mixture of o-hydroxy acetophenone (0.01 M) and 2-chlorobenzaldehyde (20% 0.04 M) was dissolved in 10 ml ethanol. To this mixture 10 ml of 40% KOH was added, the reaction mixture was stirred and kept at RT for 24 hr. Then the reaction mixture was poured over crushed ice and contents were acidified with concentrated hydrochloric acid. Chalcone thus obtained was purified by recrystallization from ethanol.

Step-2: A mixture of 2.77 gm of chalcone (10 mmol), 0.7 gm of Hydroxylamine hydrochloride (10 mmol) and 1.4 gm of anhydrous potassium carbonate (10 mmol) in 50 ml of ethanol was refluxed for 8 hours, then left to cool. The reaction mixture was poured into cold water, and the solid product was filtered off, washed with water, dried and finally crystallized from ethanol to afford Benzisoxazole derivative.

Synthesis of [3-[2-(2, 4-dichlorophenyl) ethenyl]-1, 2 benz-isoxazole] (4c)

Step-1: A mixture of o-hydroxy acetophenone (0.01 M) and 2,4-dichlorobenzaldehyde (20% 0.04 M) was dissolved in 10 ml ethanol. To this mixture 10 ml of 40% KOH was added, the reaction mixture was stirred and kept at RT for 24 hr. Then the reaction mixture was poured over crushed ice and contents were acidified with concentrated hydrochloric acid. Chalcone thus obtained was purified by recrystallization from ethanol.

Step-2: A mixture of 2.77 gm of chalcone (10 mmol), 0.7 gm of Hydroxylamine hydrochloride (10 mmol) and 1.4 gm of anhydrous potassium carbonate (10 mmol) in 50 ml of ethanol was refluxed for 8 h, then left to cool. The reaction mixture was poured into cold water and the solid product was filtered off, washed with water, dried and finally crystallized from ethanol to afford Benzisoxazole derivative.

Synthesis of [4-2-(1, 2-benzisoxazol-3-yl) ethenyl]-N, N-dimethylaniline] (4d)

Step-1: A mixture of o-hydroxy acetophenone (0.01 M) and p-dimethylamino benzaldehyde (20% 0.04 M) was dissolved in 10 ml ethanol. To this mixture 10 ml of 40% KOH was added, the reaction mixture was stirred and kept at RT for 24 hr. Then the reaction mixture was poured over crushed ice and contents were acidified with concentrated hydrochloric acid. Chalcone thus obtained was purified by recrystallization from ethanol.

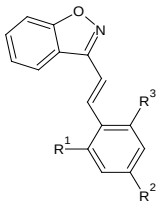
Step-2: A mixture of 2.77 gm of chalcone (10 mmol), 0.7 gm of Hydroxylamine hydrochloride (10 mmol) and 1.4 gm of anhydrous potassium carbonate (10 mmol) in 50 ml of ethanol was refluxed for 8 h, then left to cool. The reaction mixture was poured into cold water, and the solid product was filtered off, washed with water, dried and finally crystallized from ethanol to afford Benzisoxazole derivative.

Synthesis of [3-[2-(4-fluorophenyl) ethenyl]-1,2-benzisoxazole] (4e)

Step-1: A mixture of o-hydroxy acetophenone (0.01 M) and 4-flurobenzaldehyde (20% 0.04 M) was dissolved in 10 ml ethanol. To this mixture 10 ml of 40% KOH was added, the reaction mixture was stirred and kept at RT for 24 hr. Then the reaction mixture was poured over crushed ice and contents were acidified with concentrated hydrochloric acid. Chalcone thus obtained was purified by recrystallization from ethanol.

Step-2: A mixture of 2.77 gm of chalcone (10 mmol), 0.7 gm of Hydroxylamine hydrochloride (10 mmol) and 1.4 gm of anhydrous potassium carbonate (10 mmol) in 50 ml of ethanol was refluxed for 8 h, then left to cool. The reaction mixture was poured into cold water, and the solid product was filtered off, washed with water, dried and finally crystallized from ethanol to afford Benzisoxazole derivative. The Physical characteristics of all the synthesized compounds are listed in table 1.

Table 1: Physical characterization data of all the synthesized compounds



Compound	R	Molecular formula	Molecular weight	Yield (%)	m. p.(°C)	*Rf value
4a	R^1-H, R^2-H, R^3-H	$C_{15}H_{11}NO$	221.259	45	110-114	0.44
4b	R^1-Cl, R^2-H, R^3-H	$C_{15}H_{10}ClNO$	255.704	52	95-97	0.55
4c	R^1-H, R^2-Cl, R^3-Cl	$C_{15}H_9Cl_2NO$	290.149	58	78-80	0.68
4d	R^1-H, R^2-$N(CH_3)_2$, R^3-H	$C_{17}H_{16}N_2O$	264.328	55	92-95	0.52
4e	R^1-H, R^2-F, R^3-H	$C_{15}H_{10}FNO$	239.249	60	83-87	0.62

* Solvent system for TLC-toluene: chloroform: methanol (9:3:1)

[3-[2-phenylethenyl]-1, 2-benzisoxazole] (4a)

IR (KBr Vmax cm^{-1}): Aromatic C-H str at 3253 cm^{-1}, C=C str at 1454 cm^{-1}, C=N str at 1603 cm^{-1}, C-O str at 1222 cm^{-1}. ^{1}HNMR($CDCl_3$) δ ppm: 7.0-8.3 (Ar-H, 9H), 3.5-5.3 (olefinc-H, 2H), 1.5-2.9 (cyclic-H, 2H).

[3-[2-(2-chlorophenyl) ethenyl]-1,2-benzisoxazole] (4b)

IR (KBr Vmax cm^{-1}): Aromatic C-H str at 3261 cm^{-1}, C=C str at 1482 cm^{-1}, C=N str at 1604 cm^{-1}, C-O str at 1225 cm^{-1}, C-Cl str at 754 cm^{-1}. ^{1}HNMR($CDCl_3$) δ ppm: 7.0-8.4 (Ar-H, 8H), 3.8-5.6 (olefinc-H, 2H), 1.6-2.7 (cyclic-H, 2H).

[3-[2-(2, 4-dichlorophenyl) ethenyl]-1, 2 benzisoxazole] (4c)

IR (KBr Vmax cm^{-1}): Aromatic C-H str at 3270 cm^{-1}, C=C str at 1526 cm^{-1}, C=N str at 1609 cm^{-1}, C-O str at 1225 cm^{-1}, C-Cl str at 737 cm^{-1}.^{1}HNMR($CDCl_3$) δ ppm: 7.0-8.4 (Ar-H, 7H), 3.8-5.6 (olefinc-H, 2H), 1.6-2.7 (cyclic-H, 2H).

[4-2-(1, 2-benzisoxazol-3-yl) ethenyl]-N, N-dimethylaniline] (4d)

IR (KBr Vmax cm^{-1}): Aromatic C-H str at 3254 cm^{-1}, C=C str at 1482 cm^{-1}, C=N str at 1603 cm^{-1}, C-O str at 1222 cm^{-1}, C-N str at 1328 C-H str (in CH_3) at 2905 cm^{-1}. ^{1}HNMR($CDCl_3$) δ ppm: 7.0-8.2 (Ar-H, 8H), 3.8-5.6 (olefinc-H, 2H), 1.6-2.7 (cyclic-H, 2H).

[3-[2-(4-fluorophenyl)ethenyl]-1,2-benzisoxazole] (4e)

IR (KBr Vmax cm^{-1}): Aromatic C-H str at 3225 cm^{-1}, C=C str at 1482 cm^{-1}, C=N str at 1604 cm^{-1}, C-O str at 1225 cm^{-1}, C-F str at 1324 cm^{-1}. ^{1}HNMR($CDCl_3$) δ ppm: 7.0-8.3 (Ar-H, 8H), 3.8-5.6 (olefinc-H, 2H), 1.6-2.7 (cyclic-H, 2H).

***In vitro* anti-inflammatory activity [9, 10]**

Anti-inflammatory activities of the synthesized analogs were carried out by using Human Red Blood Cell (HRBC) membrane stabilization method at doses of 100 µgm, 250 µgm, and 500 µgm. To the control test tube added 1 ml solvent, 2 ml phosphate buffer, 1 ml hyposaline and 0.5 ml of HRBC suspension. To the test tube add 1 ml of the test compound, 2 ml of phosphate buffer, 1 ml of hyposaline and 0.5 ml of HRBC suspension. The assay mixture was incubated at 37 °C for 30 min and centrifuged at 3000rpm. The hemoglobin content in the supernatant solution was estimated by measuring the absorbance at 500 nm. Diclofenac sodium (100µgm/ml) was used as the standard drug. The control group was given Dimethyl sulphoxide (DMSO). Percentage inhibition of hemolysis can be calculated by the following formula,

$$\% \text{ Inhibition of haemolysis} = 100\ (OD_1 - OD_2) / OD_1$$

Where,

OD_2 = optical density of sample

OD_1 = optical density of control

The results were analyzed for statistical significance by one-way ANOVA followed by Dunnett's test and reported in table 2.

Antibacterial activity [8]

Antibacterial activity of the synthesized compounds was assessed by cup plate method. All the five synthesized analogs of Benzisoxazole were evaluated for *in vitro* antibacterial activity against *Escherichia coli* (Gram–ve) and *Staphylococcus auerus* (Gram+ve). Nutrient agar (Hi-media) was used for culturing the bacteria. The sample solutions were prepared in chloroform. The concentrations used for antibacterial screening were 100, 250, 500 µg/ml. Standard drug solution of Gentamicin (100µg/ml) was prepared in distilled water. Using a sterile cork borer of about 5 mm diameters, 5 wells were made in each Petri dish. Numbers were marked on the bottom of Petri dish to identify each cup. The sample solutions, standard solution and the vehicle control (chloroform) were placed in each cup of each Petri dish and incubated at 37±0.5 °C for 24 h. The presence of a definite zone of inhibition of any size observed and compared with standard drug and is given in table 3.

Table 2: *In vitro* anti-inflammatory activity of synthesized analogues*

Compound	Concentration (µg/ml)	Mean absorbance	% inhibition
Control	-	1.710+0.0351	-
Diclofenac sodium	100	0.655+0.0130	61.11
4a	100	0.717+0.0209	58.07
	250	0.462+0.0141	72.98
	500	0.228+0.0102	86.66
	100	0.743+0.0321	56.54
4b	250	0.642+0.0127	62.45
	500	0.335+0.0133	80.40
	100	0.841+0.0382	50.81
4c	250	0.657+0.0126	61.57
	500	0.361+0.0140	78.88
	100	0.821+0.0367	51.98
4d	250	0.524+0.0198	69.35
	500	0.301+0.0138	82.39
	100	0.721+0.0212	58.61
4e	250	0.462+0.0141	72.98
	500	0.276+0.0101	83.85

*Data expressed as mean±SEM. (n=4) and results considered significant when P< 0. 01.

Table 3: Antibacterial activities of synthesized analogs

Compound	Concentrations (µg/ml)	Zone of inhibition (mm)	
		S. aureus	E. coli
4 °	100	10	8
	250	12	10
	500	13	12
4b	100	12	10
	250	14	12
	500	15	14
4c	100	8	7
	250	10	9
	500	12	10
4d	100	12	12
	250	13	14
	500	15	15
4e	100	12	8
	250	14	9
	500	15	10
Gentamycin	100	16	16
Control	-	-	-

RESULTS AND DISCUSSION

The 1,2 Benzisoxazole analogs were synthesized using conventional synthetic methods, further recrystallized using ethanol and checked the purity by thin layer chromatography. Characterizations of the derivatives were carried out by melting point, Rf value, FTIR and ^{1}HNMR.

FTIR spectra of all synthesized compounds show absorbance bands at range 3225-3270 cm^{-1} associated with Ar C-H str, bands at 1603-1609 cm^{-1} for C-N str and bands at 1222-1225 cm^{-1} for C-O str. The ^{1}HNMR spectrum of synthesized compounds exhibit peaks in the range of 7.0-8.4 (Ar H) and 3.5-5.6 (olefinic H) of benzisoxazole.

The results of *in vitro* anti-inflammatory activity of test compounds given in table show that compounds 4a and 4e showed significant anti-inflammatory activity similar to that of Standard drug Diclofenac sodium. The results of antibacterial activity of test compounds given in table shows that compounds 4b and 4d showed significant antibacterial activity against *staphylococcus aureus* and *Escherichia coli*.

CONCLUSION

A series of 1,2-Benzisoxazole derivatives were successfully synthesized and characterized. The compounds were further screened for *in vitro* anti-inflammatory and antibacterial activity. Compounds 4a and 4e showed significant anti-inflammatory activity similar to that of Standard drug Diclofenac sodium. Compounds 4b and 4d exhibited significant antibacterial activity against *staphylococcus aureus*, and *Escherichia coli* on compared with standard drug Gentamycin. The analogs can be subjected to further detailed studies for consideration as drug candidates.

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CONFLICT OF INTERESTS

Declare none

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