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DESIGN, SYNTHESIS AND SCREENING OF BENZOXAZOLE DERIVATIVES FOR ANTIHYPERGLYCAEMIC ACTIVITY

Singh Rambir[ab,c]* and Nagori B.P.[c]

[a]Department of Pharmacy, Maharishi Arvind University, Jaipur-302012, India
[b]Department of Pharmacy, Rajasthan University of Health Sciences, Jaipur-302033, India
[c]Lachoo Memorial College of Science and Technology (Pharmacy Wing),
Sector A, Shastri Nagar, Jodhpur-342003, India

Abstract

Benzoxazole nucleus containing heterocyclic compounds plays an important role in medicinal chemistry and exhibit wide range of biological activities such as antihyperglycaemic, antidepressant, antibacterial, antifungal, antiinflammatory, antispasmodic, antiallergic and antiasthemetic activity. The present study describes the design, synthesis and in vivo testing of new series of benzoxazole derivatives as antihyperglycaemic agents in alloxan induced diabetic Wistar albino rats pioglitazone used as a standard drug. Most of the compounds showed significant antihyperglycaemic activity when compared with the standard drug pioglitazone. The structures of all synthesized compounds were determined by their IR, ^{1}H NMR and MASS spectral analysis.

Keywords: Benzoxazole, Pioglitazone, Alloxan, antihyperglycaemic activity.

INTRODUCTION

Diabetes mellitus is characterized by derangement in carbohydrate, protein, and fat metabolism caused by complete or relative insufficiency of insulin secretion and/or insulin action [1]. Diabetes mellitus (DM), long considered a disease of minor significance to world health, is now taking its place as one of the main threats to human health in the 21st century. It is the most common non-communicable disease worldwide and the fourth to fifth leading cause of death in developed countries [2-7]. Diabetes mellitus is a disease of the endocrine pancreas characterized by a relative or absolute deficiency in insulin secretion [8,9]. Type 2 diabetes mellitus is more common than type 1 diabetes and the prevalence of the type 2 diabetes increase with age [10-14]. Diabetes mellitus (DM) is one of the most challenging health problems of 21st century and is now a global epidemic with devastating humanitarian, social and economic consequences. Type 2 diabetes mellitus is the commonest form of diabetes and accounts for over 90% of diabetes mellitus [15-16]. It is the most common non-communicable disease worldwide and the fourth to fifth leading cause of death in developed countries [17]. The global figure of people with diabetes is set to rise from the current estimate of 150 million to 220 million in 2010 and 300 million in 2025 [18-20].

In the present work, benzoxazole derivatives were designed and synthesized by studying the pharmacophoric pattern of well-established and structurally different

antihyperglycaemic drugs. Strategy for synthesis was planned in such a way that the structures of synthesized compounds possess all the pharmacophoric elements; lipophilic tail, central aromatic ethereal linkage, acidic head and linkers required for antihyperglycaemic activity [21,24].

MATERIALS AND METHODS

Chemicals and Instruments

All reagents, solvents and catalyst were of LR grade and purchased from Loba Chemie Ltd., Mumbai 400 005, India. The melting point were determined in open capillary tubes and are uncorrected. The Purity of the compounds were checked on Silica gel-G coated plates were used for TLC, spots were visualized by exposure of iodine vapour in an iodine chamber. The structures of all the new synthesized compounds were confirmed by spectral analysis using IR; model Bruker alpha-T, ^{1}H NMR; AV-II 400 and MASS; Quattro IIQ- TOF MS ES. This research work deal with design & synthesize of new benzoxazole derivatives by incorporating thiazolidinedione moiety in benzoxazole framework and screen them antihyperglycaemic activity.

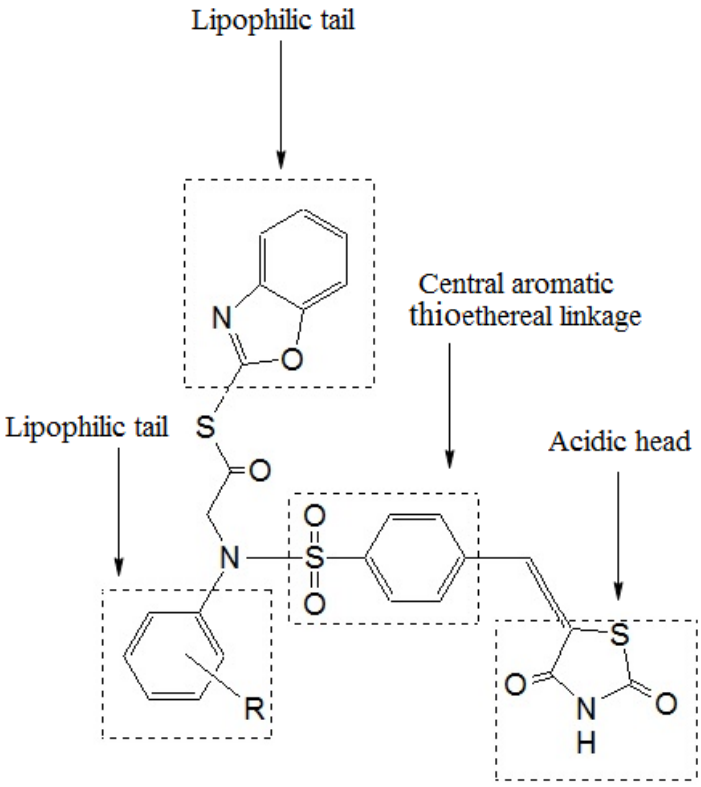

Figure 1: Designed benzoxazole derivatives

Based on design, the proposed compounds were synthesized. 2-Aminophenol was reacted with carbon disulphide to synthesize 2-mercaptobenzoxazole **(Step-I)**; which was further reacted with chloroacetyl chloride to get 1-chloroacetyl-2-mercaptobenzoxazole **(Step-II)**; chloroacetic acid was reacted to thiourea to get 2,4-thiazolidinedione,which was further

reacted with benzaldehydes to get 5-benzylidene-2,4-thiazolidinedione **(Step-III)**; this benzylidene-2,4-thiazolidinedione was further reacted with chlorosulphonic acid to get 4'-chlorosulphobenzylidine-2,4-thiazolidinedione (**Step-IV);** then 1-chloroacetyl-2-mercaptobenzoxazole was reacted with different substituted aromatic amine to get substituted *S*-1,3-benzoxazol-2-yl (phenylamino) ethanethioate [total 23 compounds (2MB-2-1 to 2MB-2-23)] **(Step-V).** These substituted *S*-1,3-benzoxazol-2-yl (phenylamino) ethanethioate compounds were further reacted with 4'-chlorosulphobenzylidine-2,4-thiazolidinedione to get their substituted *S*-benzo[d]oxazol-2-yl 2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)-N-phenylphenylsulfonamido) ethanethioate **(Step-VI)** [total 23 compounds (BD-1 to BD-23)] derivatives.

Percentage yield, melting point ranges and Rf values of synthesized compounds were determined and their structures were confirmed by spectroscopic data. **Table no.1** was showed percentage yield, m.p. range and Rf values of synthesized compounds.

Table 1: Percentage (%) yield, m.p. range and Rf values of synthesized compounds

S. No.	Compound	Percentage (%) Yield	M.P. Range (^{0}C)	Rf value
1.	2MB	77.71%	190-193	0.84
2.	2MB-1	78.35%	110-112	0.65
3.	TZD-1	92.89%	240-242	0.61
4.	TZD-2	66.61%	181-182	0.69
5.	2MB-2-1	72.08%	117-118	0.64
6.	2MB-2-2	75.42%	124-126	0.61
7.	2MB-2-3	73.73%	129-130	0.76
8.	2MB-2-4	68.01%	139-140	0.81
9.	2MB-2-5	59.16%	161-163	0.72
10.	2MB-2-6	69.64%	154-155	0.78
11.	2MB-2-7	72.52%	173-175	0.68
12.	2MB-2-8	59.74%	189-190	0.66
13.	2MB-2-9	66.18%	184-185	0.71
14.	2MB-2-10	59.48%	144-145	0.56
15.	2MB-2-11	67.20%	159-160	0.50
16.	2MB-2-12	58.52%	132-133	0.60
17.	2MB-2-13	43.36%	204-205	0.54
18.	2MB-2-14	64.52%	196-197	0.75
19.	2MB-2-15	57.18%	211-212	0.70
20.	2MB-2-16	75.84%	198-200	0.57
21.	2MB-2-17	53.36%	227-228	0.87

22.	2MB-2-18	44.20%	246-248	0.67
23.	2MB-2-19	61.58%	266-267	0.73
24.	2MB-2-20	78.04%	178-179	0.69
25.	2MB-2-21	59.62%	145-147	0.55
26.	2MB-2-22	67.50%	159-161	0.51
27.	2MB-2-23	56.46%	188-189	0.53
28.	BD-1	64.72%	251-252	0.75
29.	BD-2	71.63%	210-211	0.77
30.	BD-3	78.36%	274-275	0.66
31.	BD-4	52.48%	219-221	0.64
32.	BD-5	58.13%	236-237	0.48
33.	BD-6	65.17%	206-207	0.75
34.	BD-7	77.24%	267-269	0.77
35.	BD-8	73.79%	212-214	0.67
36.	BD-9	61.96%	297-298	0.81
37.	BD-10	72.31%	233-234	0.68
38.	BD-11	65.91%	283-284	0.64
39.	BD-12	62.97%	272-273	0.73
40.	BD-13	69.63%	292-293	0.52
41.	BD-14	74.42%	295-296	0.78
42.	BD-15	71.38%	278-279	0.74
43.	BD-16	62.96%	259-260	0.65
44.	BD-17	67.08%	309-310	0.86
45.	BD-18	68.68%	241-243	0.69
46.	BD-19	75.42%	213-214	0.82
47.	BD-20	63.63%	263-264	0.74
48.	BD-21	72.28%	224-225	0.56
49.	BD-22	81.50%	307-308	0.59
50.	BD-23	57.53%	322-323	0.53

BIOLOGICAL SCREENING OF SYNTHESIZED COMPOUNDS[21-25]

Toxicity Study

Acute toxicity study was carried out as per the procedure given in OECD Guideline No. 420. The study was conducted as follows:

Healthy young adult females nulliparous and non-pregnant Wistar albino rats (150 to 300 g) were selected for the study. The animals were randomly selected and kept in their cages for at least 5 days prior to the start of dosing, to allow for acclimatisation to the laboratory conditions.

All the experimental study was conducted as per the Regulations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The protocol of the experimental study on animals were reviewed and approved by the Local Institutional Animal Ethics Committee (Approval number 01/IAEC/2013).

The synthetic compounds were administered in a constant volume (not to be exceeding 2 mL/100g body weight of rats) over the range of doses to be tested by varying the concentration of the dosing preparation. Doses were prepared shortly prior to administration.

The synthetic compounds were administered in a single dose by gastric gavage using a oral feeding needles and rats were fasted prior to dosing (e.g. with the rat, food but not water should be withheld over-night).

The starting dose for the toxicity study were selected from the fixed dose levels of 5, 50, 300 and 2000 mg/kg as a dose expected to produce evident toxicity based, on evidence from *in vivo* and *in vitro* data from the same chemical and structurally related chemicals. A period of at least 24 hours were allowed between the dosing of each animal. All animals were observed for 14 days.

A total of five rats of one sex were used for each dose level. The one rat from each group was selected for sighting study and remaining rats were used for main toxicity study as per OECD guideline.

The time interval between dosing at each level was determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose were delayed until one was confident of survival of the previously dosed animals.

Rats were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. The toxic reactions, time of onset and length of recovery period was determined in each rat. Changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour pattern with tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma were observed in each rat. All test animals (including those that die during the test) were subjected to gross necropsy at the end of the study.

Results of acute toxicity study of different synthetic compounds of benzoxazole derivatives showed no sign of toxicity with doses of 5, 50, and 300 mg/kg, while 2000 mg/kg dose of different synthetic compounds of benzoxazole derivatives showed sign of toxicity and mortality. Convulsion, pilo-erection, hypo activity, shallow deep respiration, very weak heart beats and mortality were observed in animal treated with 2000 mg/kg dose of synthetic compounds. Post mortem examination revealed general congestion of all internal organs particularly the lung and heart. The heart was flabby engorged with blood, which may indicate heart failure due to edema.

According to OACD guidelines all the doses of 2000 mg/kg of different synthetic compounds of benzoxazole derivatives fall in category-5 and considered to be toxic. While 5, 50, and 300 mg/kg doses of different synthetic compounds fall in category-4 (nontoxic). Therefore, the LD_{50} of these synthetic compounds were found to be more than 300 mg/kg body weight. It indicates that the derivatives of benzoxazole having the cut off LD_{50} near to 500 mg/kg as per OECD guidelines no 420.

Therapeutic range was considered between 1/20 to 1/4 times of LD_{50} for any synthetic compound. Accordingly, 50 mg/kg doses of different synthetic compounds of benzoxazole derivatives were selected for antihyperglycaemic activity in alloxan induced diabetic rats.

Antihyperglycaemic Activity in Alloxan Induced Diabetes rats

The synthetic compounds were evaluated for their *in-vivo* antihyperglycaemic activity in albino Wistar rats using alloxan induced diabetes method.

Adult male and female albino Wistar rats weighing between 150 and 300 g were used in this study. All the experimental study was conducted as per the Regulations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

The animals were housed in clean polacrylic cages (38x23x10 cm) with not more than five animals per cage and they were acclimatized under standard laboratory conditions (temperature 25±2°C) and light cycle (12 h light and 12 h dark). All animals were fed with standard pellet diet and water *ad libitum*.

The doses of synthetic compounds were taken as 50 mg/kg of body weight. The selection of doses of synthetic compounds was selected on the basis of toxicity study conducted on rats as per OECD guideline. Doses were prepared in normal saline shortly prior to administration.

The synthetic compounds were administered in a single dose by gastric gavage using a oral feeding needles and rats were be fasted prior to dosing (e.g. with the rat, food but not water should be withheld over-night).

Diabetes was artificially induced by intraperitonially injection of freshly prepared solution of alloxan in 0.1 M citrate buffer that's P^H was adjusted to 4.5 and temperature of the solution was maintained in between 2 to 8 ^{0}C at the dose of 110 mg/kg body weight to overnight fasted rats. The blood samples of rats were collected through snipping the tail vein. The diabetic status of rats were confirmed post 48 hr of alloxan injection by monitoring blood glucose level with the help of Glucometer. The rats which having blood glucose levels more than 200 mg/dl were considered as diabetic and selected for the study.

130 Healthy albino Wistar rats were marked and randomly distributed into 26 groups of 5 animals in each group. All the animals were fasted overnight. The groups were divided as follows:

Group (I):- Normal control Group (normal rats were received vehicle only)

Group (II):-Diabetic control Group (alloxan induced diabetic rats were received vehicle only)

Group (III) to (XXV):- Test Groups (Alloxan induced diabetic rats were received synthetic compounds BD-1 to BD-23 in a dose of 50 mg/kg).

(XXVI):- Standard Group (Alloxan induced diabetic rats were received pioglitazone as a standard drug in a dose of 30 mg/kg).

The blood sample of rats were collected through snipping the tail vein. Blood sample were collected at day 0, 3, 7 and 14 for all 26 groups of rats. The blood samples were collected in micro centrifuge tubes (Eppendorf tubes) and centrifuge for 10 minutes at 10,000 rpm. Blood glucose level was estimated with the help of Glucometer. The blood glucose level was expressed as mg/dl of blood.

All the results were expressed as mean±SEM. The data obtained in the study was subjected to one way analysis of variance (ANOVA) followed by dunnett test for determining the significance. P-value <0.05 was to be considered for statistical significant.

One way analysis of variance (ANOVA) followed by dunnett test. Value are expressed as mean ± SEM; n= number of animals. P>0.05 was considered as non-significant, *P<0.05 was considered as significant. **P<0.005 was considered highly significant, ***P<0.0005 was considered as very highly significant.

RESULTS AND DISCUSSION

All the synthesized compounds of benzoxazole derivatives were screened for their antihyperglycaemic activity against alloxan induced diabetic control rats, Pioglitazone were used as a standard drug. The screening results revealed that the compounds BD-5, BD-6, BD-10, BD-12, and BD-14 exhibited highest hypoglycemic activity while compounds BD-1, BD-2, BD-4, BD-7, BD-8, BD-9, BD-11, BD-13, BD-15, BD-16, BD-17, and BD-2 exhibited moderate hypoglycemic activity. Compounds BD-3, BD-18, BD-19, BD-21 and BD-23 exhibited weak hypoglycemic activity and compounds BD-22 was not more effective to lowering blood glucose level. The results of antihyperglycaemic activity of all test compounds are depicted in table no.2.

Table 2: Effect of benzoxazole derivatives on blood glucose level after daily administration for 14 days

Groups and Treatment (n=5)		Blood glucose level (mg/dl)			
		Day 0	Day 3	Day 7	Day 14
I.	Normal control	107.9±3.87	108.92±3.11	110.84±2.98	111±3.64
II.	Diabetic control	258.2±6.72	252.8±6.28	251.6±4.87	259±8.40
III.	BD-1	247.6±12.23	224.6±8.68	183.4±3.90**	133.2±2.31***
IV.	BD-2	241.8±7.57	217±4.74*	173.4±4.15***	132.2±2.24***
V.	BD-3	227.8±2.69	210.8±6.71	197.2±6.93*	140.6±4.55***
VI.	BD-4	237.6±6.37	227.8±6.55*	202.2±9.41	130.4±1.02***
VII.	BD-5	246±11.86	222.2±7.69	178.8±3.18*	127.4±2.13***
VIII.	BD-6	244.4±7.37	217±4.78	172.6±4.03**	127.4±1.50***
IX.	BD-7	233.2±3.42	213.6±7.82	185±13.10*	133±1.48***

X.	BD-8	241±3.11	225.4±4.15*	184.8±5.38***	134.6±4.43***
XI.	BD-9	247.2±6.35	221.4±8.60	185±5.02*	135.4±1.07***
XII.	BD-10	238±9.46	217.2±4.69	173.2±6.06**	126.6±1.86***
XIII.	BD-11	235.4±6.4	223.4±5.39*	187.6±9.19***	138.2±3.92***
XIV.	BD-12	237.2±3.55	225.2±3.97	190.8±8.54*	123.8±2.47***
XV.	BD-13	251.6±11.85	226±4.64	176.6±3.15*	132.6±2.13***
XVI.	BD-14	242±6.72	216.4±4.77*	161.2±2.93***	120±2.04***
XVII.	BD-15	255.4±7.21	227.6±2.85	183±8.37*	133.6±2.73***
XVIII.	BD-16	250.6±5.67	226.2±2.41	206±5.67**	135.4±2.76***
XIX.	BD-17	247.6±5.25	224.6±6.31**	184±5.10***	137.2±2.59***
XX.	BD-18	246.8±4.21	224.4±2.24	182.4±4.83*	143.4±2.37***
XXI.	BD-19	238.8±1.56	224.5±1.5	184.5±6.95**	143±2.12***
XXII.	BD-20	254.8±5.03	233±2.16*	171.8±5.84***	138.2±1.77***
XXIII.	BD-21	258.8±8.81	225.4±1.91	179.8±5.51**	145.8±2.98***
XXIV.	BD-22	246.6±9.08	228.2±5.85	188.6±6.38*	152.8±2.47***
XXV.	BD-23	236±2.73	219.4±3.86**	178.8±8.62***	140.4±2.13***
XXVI.	Standard Drug (Pioglitazone)	253±7.85	194±6.76**	126±2.19***	101.6±0.67***

One way analysis of variance (ANOVA) followed by dunnett test. Value are expressed as mean ± SEM; n= number of animals. P>0.05 was considered as non-significant, *P<0.05 was considered as significant. **P<0.005 was considered highly significant, ***P<0.0005 was considered as very highly significant.

CONCLUSION

In the present work, benzoxazole derivatives were designed and synthesized by studying the pharmacophoric pattern of well-established and structurally different antihyperglycaemic drugs and evaluated for antihyperglycaemic activity. The compounds BD-5, BD-6, BD-10, BD-12, and BD-14 exhibited highest hypoglycemic activity and compounds BD-22 was not more effective to lowering blood glucose level. It was concluded that N,N-substituted aniline ring having *orhto* and *para* substitute as methyl, ethyl methoxy and ethoxy and di-ortho substituted methyl group, these compound were showed maximum antihyperglycaemic activity in alloxan induced diabetic albino Wistar rats. This investigation thus indicates the importance of these benzoxazole derivatives as potential lead candidates.

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