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BIOLOGICAL ASSESSMENTS OF SUBSTITUTED BENZOTHIAZOLE DERIVATIVES IN STREPTOZOCIN INDUCED DIABETES RATS

Sunil Kumar[1, 2], Abhilasha Mittal [1], Ashish Pathak[2], Sanjeev K. Sahu[3*]
[1]NIMS Institute of Pharmacy, NIMS University, Jaipur (Rajasthan) INDIA
[2]Ravishankar College of Pharmacy, Bhopal (M.P.) INDIA
[3]School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, INDIA

Abstract

Benzothiazole is a key pharmacophore which was associated with in diverse biologically activities resulted in the new drugs for the different classes. Diabetes mellitus (DM) is chronic metabolic disorder which can be induce in the experimental model using streptozocin (STZ) /alloxan for the evaluation of antidiabetic activity. The present research work were reported the biological assessment of some synthesized benzothiazole derivatives 6(a-i) and 7(a-j) in the streptozocin induced diabetes rat model and among them two compounds 6f & 7d exhibited more potent anti-diabetic activity at 350 mg/kg p.o.
Keywords : Diabetes Mellitus (DM), Benzothiazole, Streptozocin, Antidiabetic Activity

Introduction

Streptozocin (STZ) is a glucosamine-nitrosourea compound that has been in clinical trial since 1967. STZ is the most commonly used drug for induction of diabetes in rats [1-7]. It offers the economical effective and efficient method which is commonly used in the most strains of rodents [8-15]. Intra-venous injection of 60mg/kg dose of streptozotocin in adult wistar rats causes swelling of pancreas followed by degeneration of Langerhans islet beta cells and induces experimental diabetes mellitus in the 2-4 days. Three days after degeneration of beta cells, diabetes was induced in all animals. Nicotinamide-adenine dinucleotide (NAD) in pancreas islet beta cells and causes histopathological effects in beta cells which probably intermediates induction of diabetes [16-20].

The currently available drugs have a number of side effects and thus searching for a new class of compounds is crucial to overcoming these problems. Heterocyclic compounds are the mainstay of antidiabetic therapy for many years. Benzothiazole is a weak heterocyclic base, having varied biological activities and of great scientific interest nowadays [21]. Benzothiazole derivatives containing benzimidazole and imidazoline ring have diverse chemical reactivity along with a broad spectrum of biological activity. There was the significant interest to synthesise the benzo-thiazole derivatives with an aim to obtain potent biologically active and safe anti-diabetic agents [22, 23].

Materials and Methods

We were already synthesized and reported the different substituted Benzthiazole derivatives 6(a-i) and 7(a-j) listed in Table 1 using following scheme [24, 25]:

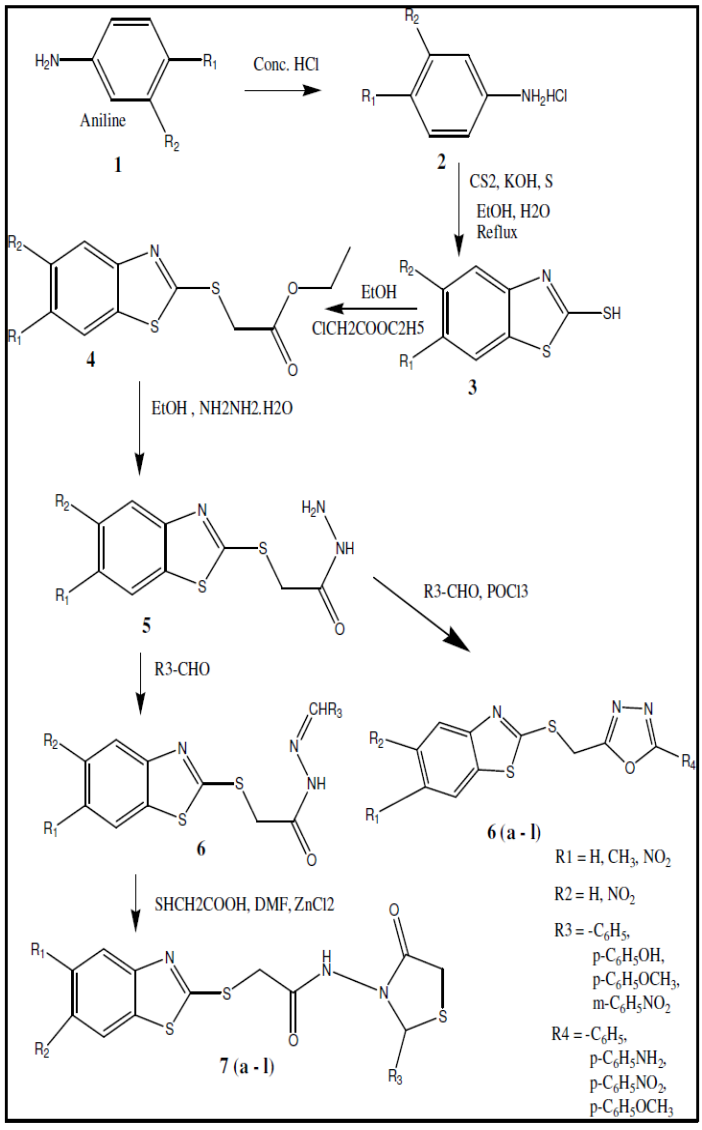

Table 1: List of Benzothiazole Derivatives with Different Substitutions

S. NO.	Compound Code	R_1	R_2	R_3
1.	7a	CH_3	H	C_6H_{5-}
2.	7b	CH_3	H	$pOHC_6H_{4-}$
3.	7c	CH_3	H	$pOCH_3C_6H_{4-}$
4.	7d	CH_3	H	m $NO_2C_6H_{4-}$
5.	7e	NO_2	H	C_6H_{5-}
6.	7f	NO_2	H	$pOHC_6H_{4-}$
7.	7g	NO_2	H	$pOCH_3C_6H_{4-}$
8.	7h	NO_2	H	m $NO_2C_6H_{4-}$
9.	7i	H	NO_2	C_6H_{5-}
10.	7j	H	NO_2	$pOHC_6H_{4-}$
11.	7k	H	NO_2	$pOCH_3C_6H_{4-}$
12.	7l	H	NO_2	m $NO_2C_6H_{4-}$
13.	6a	CH_3	H	C_6H_{5-}
14.	6b	CH_3	H	$pNH_2C_6H_{4-}$
15.	6c	CH_3	H	$pNO_2C_6H_{4-}$
16.	6d	CH_3	H	$pOCH_3C_6H_{4-}$
17.	6e	NO_2	H	C_6H_{5-}
18.	6f	NO_2	H	$pNH_2C_6H_{4-}$
19.	6g	NO_2	H	$pNO_2C_6H_{4-}$
20.	6h	NO_2	H	$pOCH_3C_6H_{4-}$
21.	6i	H	NO_2	C_6H_{5-}
22.	6j	H	NO_2	$pNH_2C_6H_{4-}$
23.	6k	H	NO_2	$pNO_2C_6H_{4-}$
24.	6l	H	NO_2	$pOCH_3C_6H_{4-}$

Biological Evaluation and Assessment of Synthesized Derivatives

There are mainly two types of chemically induced diabetes experimental models used for biological evaluation of antidiabetic agents.

A. Streptozocin (STZ) induced diabetes

B. Alloxan induced diabetes:

Animal model

Albino rats of Wister strain of either sex between the age of 2-3 months and weighing 150-200 grams were procured for the present study. The animals were housed in husbandry maintaining the typical conditions with 12 hours light-dark dark cycles at 25°C temperature and 40-60% humidity. They were acclimatized for the seven days by providing standard rat pellet diet with water ad libitum prior to start the study. All the animal experiment protocols were approved by CPCSEA, Institutional Animal Ethics Committee, Sapience Bio-analytical Laboratory Bhopal, Madhya Pradesh, India reg. no. 1447/PO/a/11 /CPCSEA.

Determination of acute toxicity (Kumar *et al.*, 2011) [26]

The acute toxicity of synthesized benzothiazole derivatives were determined by using female albino rats (20–25 g) which were maintained under the standard conditions. The acclimatized animals (n= 5) were kept fasting with water *ad libitum* for 12 h prior to the experiment. The animals were administered with single dose of test compounds at a dose of 2000 mg/kg and observed for their mortality during 14 days period for toxicity study. The doses were increased up to 1000 mg/kg and rats were observed up to 02 weeks for their behavioral, economical and neurological profiles except slight depression in their activity. No such signs, symptoms and mortality were observed even after 14 days. Hence the LD50 cut off value of the test compounds was fixed at 350 mg/kg and the same dose was considered for evaluation of anti diabetic activity.

Assessments of anti-diabetic activity in Streptozocin - induced diabetic rats (Nambirajan *et al*, 2018) [27]

Induction of experimental diabetes by Streptozocin (STZ)

The animals were administered with the single dose of Streptozotocin (35 mg/kg) in normal saline by intra peritoneal injection for the induction of diabetes. After Streptozotocin administration (i.p.), rats were given 5% (w/v) dextrose solution in feeding bottles for next 24 h in their cages to prevent hypoglycaemia. The animals showing blood glucose range of 200-400 mg dL^{-1} were used for the experiment and the hyperglycemia was confirmed after 72 hours of Streptozotocin monohydrate administration (i.p.). The animals were also observed for consistent hyperglycaemia (fasting blood glucose) between 200-400 mg/dl up to 14 days.

Experimental Design

Animals were divided into fifteen groups with 6 animals each (n=6) and named Group 1 Non diabetic animals received normal saline solution as normal control group; Group 2 diabetic animals received 1 ml of 0.5% carboxy methyl cellulose as positive diabetic control group; Group 3 diabetic animals received Glibenclamide 20 mg/kg as standard group; Groups (4-15) & Groups (16-27) diabetic animals received compounds (6a-6l) & (7a-7l) in a single dose of 350 mg/kg body weight p.o. respectively for 14 days.

Blood Glucose Measurement

Blood glucose level was monitored by tail dipping method. The blood glucose concentration was checked on dextrostrix reagent pad using microprocessor digital blood glucometer (Sugerchek Glucometer, Wockhardt manufacture,India). The blood glucose level was monitored at 0 hr, 7hr, 14hr, and 21hr respectively. The antidiabetic activities of synthesized compounds on diabetic rats were reported in Table 2.

Results and Discussion

All the results of antidiabetic activity of synthesized compounds 6(a-l) and 7(a-l) on diabetic rats summarized in Table 2 revealed that most of the synthesized compounds exhibited antidiabetic response at the end of twenty first day of experimental period.

Table 2: Antidiabetic activity of synthesized compounds (S1-S24) on diabetic rats

S. No.	Treatment	Blood Glucose Level (mg/dl)				% Reduction in Blood Glucose
		0th day	7th day	14th day	21st day	
1.	**Normal Control**	106 ± 0.98	104 ± 1.30	104 ± 0.98	101 ± 0.90	4.08
2.	**Diabetic Positive control**	338 ± 10.17	357 ± 2.41	344 ± 3.11	336± 6.42	0.65
3.	**Glibencl-amide 10 mg/kg**	352 ± 2.52	348 ± 3.16	243 ± 4.33	119 ± 6.59	67.30
	Each Test Group receives 350 mg/kg (p.o.) as effective dose.					
4.	6a	316 ± 1.02	303 ± 3.12	258 ± 7.82	202 ± 7.06	36.04
5.	6b	342 ±1.38	329 ± 4.03	272 ± 6.03	207 ± 7.29	39.34
6.	6c	294 ± 1.30	282 ± 1.64	237 ± 3.13	185 ± 5.18	37.10
7.	6d	286 ± 1.33	275 ± 1.47	233 ± 4.07	185 ± 2.46	35.41
8.	**6e**	**371 ± 1.08**	**353 ± 2.77**	**258 ± 1.35**	**142 ± 5.24**	**61.58**
9.	**6f**	**362 ± 1.33**	**348 ± 1.66**	**245 ± 2.34**	**126 ± 6.10**	**65.15**
10.	**6g**	**358 ± 2.16**	**349 ±1.78**	**252 ±8.40**	**128 ± 6.23**	**64.12**
11.	6h	302 ± 2.35	291 ± 1.44	218 ± 9.18	135 ± 7.85	55.25
12.	6i	331 ± 2.23	313 ± 4.99	244 ± 7.80	163 ± 6.60	50.73
13.	6j	317 ± 2.24	300± 4.38	228 ± 5.27	144 ± 3.45	54.62
14.	6k	339.66 ± 1.40	326 ±2.04	255 ± 2.68	176 ± 3.75	48.14
15.	6l	311 ± 1.30	297 ± 1.05	238 ± 2.68	168 ± 2.84	45.88
16.	7a	323 ± 2.31	305 ± 2.48	258 ± 1.48	186 ± 1.32	42.29
17.	7b	315 ±3.19	302 ± 2.83	251 ± 2.35	166 ± 1.08	47.24
18.	7c	298 ±.187	286 ± 1.68	246 ± 2.71	195 ± 1.32	34.53
19.	**7d**	**366 ± 2.76**	**346 ± 3.12**	**238 ± 1.79**	**125 ±1.01**	**65.68**
20.	7e	342 ± 4.02	225 ± 2.55	209 ± 1.41	183 ± 1.86	46.40
21.	7f	286 ± 1.45	275 ± 2.15	232 ± 3.11	175 ± 2.12	38.57
22.	**7g**	**351 ± 1.98**	**334 ± 4.07**	**268 ± 2.59**	**132 ± 1.11**	**62.17**
23.	**7h**	**331 ± 2.61**	**322 ± 2.84**	**272 ± 1.72**	**126 ± 1.52**	**61.75**
24.	7i	293 ± 1.37	284 ± 1.79	244 ± 2.49	184 ± 1.89	37.10
25.	7j	318 ± 1.69	299 ± 1.95	247 ± 2.36	152 ± 1.18	51.92
26.	7k	348 ± 3.18	329 ± 3.36	269 ± 1.88	178 ± 1.05	48.74
27.	7l	327 ± 2.98	310 ± 2.82	232 ± 3.14	134 ± 1.93	58.99

Statistical Analysis

The values were expressed as mean ± S.E.M. Data were analyzed using One-way ANOVA followed by Tukey-Kramer test. The values were considered to be significant at $p<0.05$ and $p<0.01$ level.

It has been found that oral administration of synthesised compounds specifically 6e, 6f, 6g, 6h, 7d, 7g, 7h and 7l at a defined dose of 350 mg/kg b.w. exhibited a more significant reduction in blood glucose than other compounds in diabetic rats. However, the compound 6f &7d exerted maximum glucose lowering effects whereas 6d & 7i showed minimum glucose lowering effects. This study reveals the result of test groups when significantly compared with positive control (Streptozocin 60 mg/kg) i.v. and standard Glibenclamide 10 mg/kg (p. o.).

Conclusions

The assessment of biological activity was performed for the determination of anti-diabetic activity of all the synthesized benzothiazole derivatives in a Streptozocin - induced diabetic rat model. The streptomycin was used to induce the diabetic hyperglycemia condition characterized with elevation of glucose level in plasma. It also considered as significant marker of renal dysfunction. Amongst all these synthesized derivatives compound 6f & 7d given more potent anti-diabetic activity at 350 mg/kg p. o. and would be of better use in drug development to combat the metabolic disorder in future.

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