SYNTHESIS AND *IN-VITRO* ANTI-DIABETIC EVALUATION OF SOME NOVEL β-LACTUM-BENZOTHIAZOLE DERIVATIVES

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Abstract
The present investigation is to synthesize five novel benzothiazole-azetidinone derivatives (PN1-PN5) by facile method. The synthesized compounds were characterized by **IR, NMR** spectroscope **and Mass** spectrometry respectively. Five novel derivatives were screened for invitro anti-diabetic activity by 3T3-L1 adipocytes method. All the synthesized derivatives shown equipotent activity when compared standard Insulin and Pioglitazone drug.

Keywords: Benzothiazole, Azetidinone , Anti-diabetic, Insulin , Pioglitazone

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Introduction:

BENZOTHIAZOLE

Benzothiazole[1] comprising sulfonamide pyrazole derivatives have been synthesized and evaluated for their various activities.

The sulfonamide[2-5] drugs were the first effective chemotherapeutic agents to be employed systemically for the prevention and cure of bacterial infection in human beings. The introduction of trimethaprim and sulpham-ethoxazole has resulted in increased use of sulfonamide for the treatment of specific microbial infections.Benzothiazoleswithsulphonylgroupandpyrazoleetcwerereported to possess variouspharmacological activity of clinical importance.

The chemistry and pharmacology of pyrazole[6-10] have been of great interest because pyrazole derivatives possess various biological activities like anti-bacterial anti-inflammatory, anti-TB anti-diabetics anti-funal etc.

2-AZETIDINONE[11-13]:

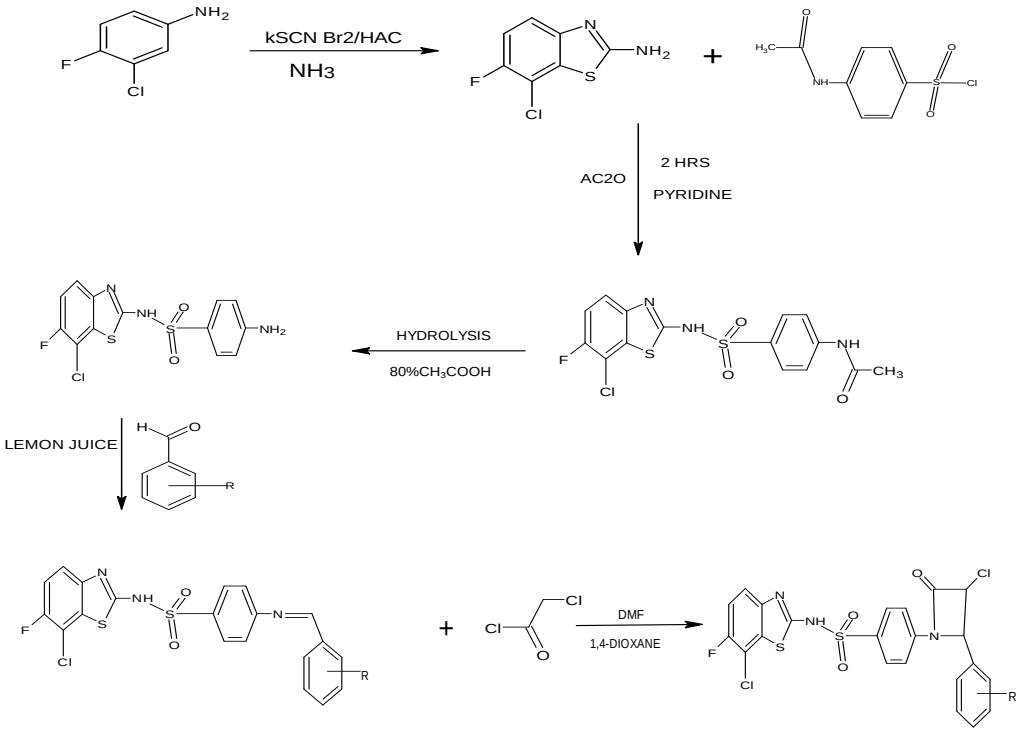
2-Azetidinones commonly known as β-lactams are well-known heterocyclic compounds among organic and medicinal chemists. The activity of famous antibiotics such as penicillins, cephalosporins, nocardicins and carbapenems are attributed to the presence of 2-azetidinone ring in them. Parent heterocyclic ring of azetidinones is azetidine. Azetidine is a 4 member heterocyclic ring system with nitrogen as hetero atom. 2-Azetidinones are also known as β-lactams and it is one of the most common heterocyclic rings found in antibiotics. 2-Azetidinones consists of a carbonyl group on the second position. Azetidinones are very important class of compounds possessing wide range of biological activities such as antimicrobial,antitumor , antitubercular , anticancer , cytotoxic ,pesticidal enzyme inhibitors , elastase inhibitors & cholesterol absorption Inhibitors. Recent years have seen a resurgence of interest in the development of stereo and enatioselective methodologies.

Due to wide range of pharamacological activities of benzothiazole and azetidinone, an attempt was made to combine both the nucleus for promising anti-diabetic activity.

Materials and methods:

Melting point was determined by open capillary tube method and is uncorrected. T.L.C was run on silica gel G plates using n-butyl alcohol, ethyl acetate and carbontertachloride (1:2:1) as developing solvents for the purity of the compounds. I.R. Spectra were recorded on Shimadzu FTIR Spectrophotometer by using KBr pellet technique.

SCHEME-I



SL.NO.	COMPOUND CODE	R
1	PN1	-H
2	PN2	*P*-Cl
3	PN3	*O*-OH
4	PN4	3-OCH_3, 4-OH
5	PN5	*P*-NO_2

METHODOLOGY:

Synthesis of 2-amino-6-fluoro-7-chloro-benzothiazole:

- 8gm (0.08mol) of potassium thiocyanate and 1.45g (0.01 mol) of fluoro chloro aniline were added to ice cold ice cold glacial acetic acid (20mL). The mixture was placed in water bath and stirred in magnetic stirrer. 1.6ml of bromine in 6ml of glacial acetic acid was added slowly to above mixture, care were taken to maintain the mixture below the room temperature. After addition of bromine the solution was stirred for 2 hours below room temperature for 10 hours, it was then allowed to stand over night, to get an orange precipitate settle at the bottom, water (6ml) was added quickly and slurry was heated at 85^0c on a steam bath and filtered hot. The orange residue was placed in a reaction flask and treated with 10ml of glacial acetic acid heated again to 85^0c and filtered hot. The combined filtrate was cooled and neutralised with concentrated ammonia solution to PH 6 A dark yellow precipitate was collected. Recrystalised from benzene, ethanol of (1:1) after treatment with animal charcoal gave yellow flakes of 2-amino-6-fluoro-7-chloro-(1,3)-benzothiazole. After drying in a oven at 80^0C, the dried product was stored in desicator, the yield was 51% and melting poing is 210-212^0c.

- **Preparation of *p*-acetamido benzene sulphonyl chloride:**

- A 500 ml two necked flask was equipped with a dropping funnel and a reflux condenser, attached the top of the later to calcium guard tube for the absorption of hydrogen chloride. 20g (0.148 mol) of dry acetanilide was placed in the flask and 50 ml (90g, 0.77 mol) of a good grade of chlorosulphonic acid in the dropping funnel. A calcium guard tube was inserted to the later. Chlorosulphonic acid was added in small portions and the contents of flask were shaken from time to time to ensure thorough mixing. When the addition has been made the reaction mixture was heated on a water bath for 1 hr in order to complete the reaction. It was allowed to cool and the oily mixture was poured with stirring into 300g of crushed ice contained in a 1 litre beaker. This operation was carefully carried out in the fume cupboard since the excess of chloro-sulphonicacid reacts vigorously with the water. The flask was rinsed with a little ice water and rinsing was added to the contents of the beaker. The mixture was stirred for several minutes, the solid lump material was broken to obtain even suspension of the granular white solid. The obtained solid *p*-acetamido benzene sulphonyl chloride was filtered at the pump and washed with cold water. It was pressed and drained well, kept for drying.

Condensation of 2-amino-6-fluoro-7-chloro-benzothiazole and *p*-acetamido benzene sulphonyl chloride

2-amino-6-fluoro-7-chloro (1,3) benzothiazole (0.013 mol) was taken in pyridine (4 ml) and acetic anhydride (20 ml), to this *p*-acetamido benzene sulphonyl chloride (0.01 mol) were added and the mixture was kept in water bath for 2 hrs. The reaction mixture then poured in to 20 ml of ice cold water. The solid obtained was filtered and recrystallized from ethanol (80%) to get pure compound 6-fluoro-7-chloro-2-(*p*-acetamido benzene sulphonamido) (1,3)- benzothiazole.

Hydrolysis of the 6-fluoro-7-substituted-2-(*p*-acetamido benzene sulphonamido) (1,3) benzothiazoles.

The derivatives obtained were then hydrolyzed by boiling them in 50 ml of 80% acetic acid for 4 to 5 hrs and the contents were poured onto crushed ice. The obtained hydrolyzed derivatives were filtered at suction and dried.

Synthesis of Schiff base by Grind stone method:

Take equimolar benzthiazole derivatives (0.01mol) and Substituted benzaldehyde derivatives (0.01mol) in to a mortar, to this add 2.5mL of lemon juice and 5ml water. The reaction mixture was grounded for 15-30 min. The reaction monitored by TLC. After completion of reaction add 25ml of water and stirred product for 5min. The separated out solid was filtered, washed with water and recrystallized from ethanol to give the corresponding Schiff bases.

Synthesis of Benzthiazole-azetidinone derivatives (PN-1 to PN-5)

To the benzthiazole Schiff base compounds (0.01mol) in absolute benzene(100 ml), add triethylamine (2-3 drops) and

chloroacetylchloride(0.02 mol) were added drop by drop with stirring the mixture for 1hr, further the reaction mixture refluxed for 1 hour .the reaction was monitored by TLC, The reaction mixture was cooled and poured into ice. The solid thus obtained was filtered and recrystallized from ethanol.

Biological Evaluation

In-vitro Antidiabetic Activity:[14-16]

Procedure:

3T3-L1 adipocytes, were seeded at a density of ~1500 cells per well in a 96-well plate, differentiated and maintained for another 10 days prior to use. To assay glucose uptake, adipocytes were starved in 100 µl serum free adipocyte medium overnight (to enhance glucose uptake) then washed with PBS, followed by a incubation (40 min) in an glucose free medium (100 µl Krebs-Ringer-Phosphate-HEPES (KRPH) buffer with 2 % BSA) then stimulated either with insulin (PGZ) (10 µM), compounds (10 µg/ml) or PBS. 10 µl of 10mM 2-Deoxy glucose (DG) was added and the cells incubated for 20 min. The amount of glucose uptake was determined as per manufactures protocol using the Glucose uptake kit from Biovision (glucose uptake colorimetric assay kit, the 2-DG6P is oxidized to generate NADPH, which can be determined by an enzymatic recycling amplification reaction, color generated can be quantified colorimetrically at 412 nm.). The calculation was carried out keeping 100% glucose uptake for Pioglitazones (PGZ) was used as a standard drug.

- ✓ 2-DG uptake = Sa/Sv (pmol/µl or nmol/ml or µM)
- ✓ Where: Sa is the amount of 2-DG6P (in pmol) in sample well calculated from Standard Curve.
- ✓ Sv is sample volume (in 20 µl) added into the sample well. antidiabetic activity of the synthesized derivatives was performed by the Glucose uptake assay and the results were tabulated below.

Results and Discussion

In-vitro Antidiabetic Activity:

Effect of compounds (PN-1 to PN-5) on 2-DG uptake in 3T3-L1 presence and absence of insulin:

S.No	Compound	OD (412)	2DG6P(pmol)	2-DGuptak(Pmol/µl)
1	Insulin (1 micro Mol)	3.04	174	11.5
2	PN 1	4.5	100	8.5
3	PN1 +Insulin	8.0	165	10.5
4	PN 2	5.5	105	9.2
5	PN2 +Insulin	9.5	169	10.2
6	PN3	3.5	60	4.0
7	PN3 +Insulin	5.0	80	6.5
8	PN-4	2.5	40	3.0
9	PN4 +Insulin	4.0	70	5.5
10	PN5	0.6	10	1.0
11	PN5 + Insulin	1.5	30	2.5
10	Pioglitazone(10Micro.Mol)+Insulin(1 Micro.Mol)	10.4	450	22.5
11	Pioglitazone (10 Micro.Mol)	3.1	210	10.5

Conclusion:

In the present research work, five novel β-lactum-benzothaiazole (PN-1-PN2) derivatives were designed, synthesized and confirmed by IR, NMR spectroscopy and Mass spectrometry. The synthesized compounds were screened for invitro-antidiabetic activity using 3T3-L1 adipocytes method. The results were compared with standard insulin and pioglitazone. The invitro activity of synthesized drug molecules was equipotent to that of repective stardards. Futher QSAR and In-vivo and toxicological studies are required to confirm for the utility of the synthesized β-lactum-benzothiazole derivatives as a potential anti-diabetic drug.

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