ISSN No. 2455-5800
Journal of Scientific Research in Allied Sciences

Original Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NEW ARYLOXYPROPANOLAMINES DERIVATIVES

Anup K. Chakraborty[1], Alka Singh[1], Parmeshwar B. Shinde[2]

1. School of Pharmacy & Research, People's University, Bhopal, M.P.
2. Modern College of Pharmacy, Nigdi, Pune, Maharashtra

Article history:
Submitted on: November 2016
Accepted on: December 2016
Email: info@jusres.com

Abstract

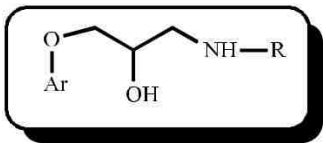
An efficient route of synthesis of some new Aryloxypropanolamine derivatives was proposed and Anti-diabetic activity. In this research paper, Aryloxypropanolamine derivatives were synthesized via three step process. In the first step, imine intermediate was obtained (S1 to S5) and in the second step, oxirane intermediates were obtained (E1 to E5) and finally Aryloxypropanolamine derivative was obtained (A1 to A5). The blood sugar level was monitored at specific interval there was a good decrease in the elevated levels of the blood glucose in 6 hours study after oral ingestion of the standard as well as test drug. The synthesized aryloxy propanolamine derivatives had shown better anti-diabetic activity. Among the five synthesized derivatives, A5 shows potent and significant anti-diabetic activity as compared to other derivatives.

Keywords: Synthesis, Aryloxypropanolamine, derivatives, anti-diabetic.

1. Introduction

Aryloxypropanolamines are an important class of β-adrenergic blocking agents (β- blockers) and extensively used in medicinal chemistry for the treatment of hypertension, diabetes, angina pectoris, glaucoma, anxiety, and obesity. Aryloxypropanolamines derivatives having anti-fat activity, anti-hypertensive activity and an anti-diabetic activity and their method of preparation and applications, particularly as human and veterinary medicine and animal food additive[1].

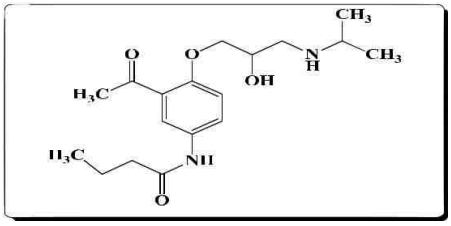
General chemical structure for Aryloxypropanolamines[2-7]



One of the most straightforward synthetic approaches for the preparation of β– blockers involves the heating of epoxides with an excess of amines at elevated temperature.

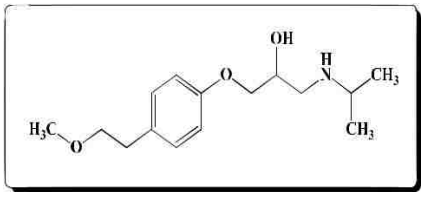
β Adrenergic blocking agents, mostly comprising of β amino alcohols, are of pharmaceutical significance and have received major attention due to their utility in the management of cardiovascular disorders including hypertension, angina pectoris, cardiac arrhythmias and other disorders related to the sympathetic nervous system. The discovery of propranolol, the first successful drug has prompted the synthesis of many thousands of compounds containing an aryloxy propanolamine moiety.

Following are the derivatives of Aryoxypropanolamines having,



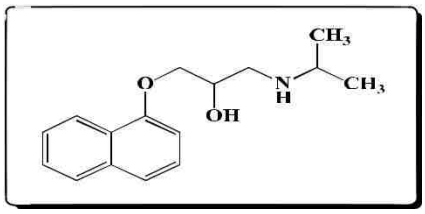
Metaprolol (2)

(Selective beta 1 blocker)



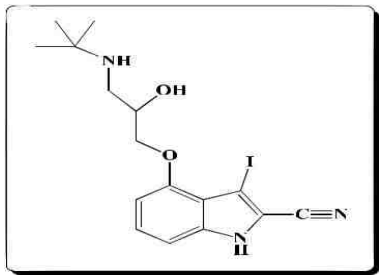
Propranolol (3)

(Beta 1/2 non-selective adrenergic antagonist)



Acebutol (4)

(slightly beta 1 selective)



Iodocynopindolol (6)

Diabetes mellitus in human is a manifestation of metabolic disturbances due to the dietary intake of excess carbohydrates and lipids (Vats et al., 2003). Hyperglycemia and hyperlipidemia are important risk factors in the development of cardiovascular diseases and metabolic disorders (Frishman, 1998). Various chemicals can be used to produce diabetes in the rodents particularly alloxan and streptozotocin which has been extensively used in diabetes research. The development of hyperglycemia following intraperitoneal injection of alloxan is primarily due to

the direct pancreatic beta cells destruction and resulting insulin deficiency (Luo et al, 1998). There is relation between diabetes and high-fat diet observed in rodents.8

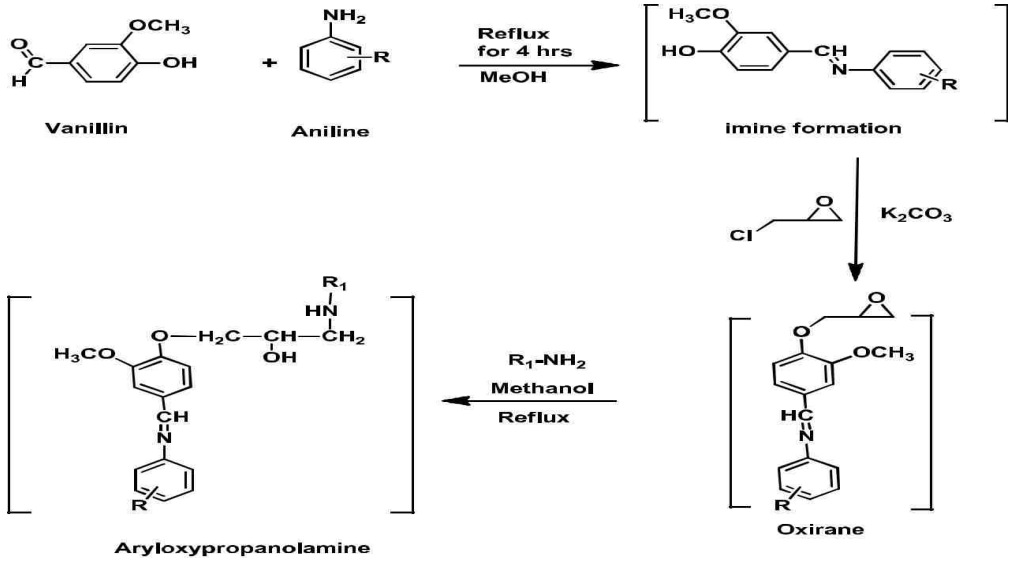
Beta 3 adrenergic receptors are found on the cell surface of both white adipose tissues (WAT) and brown adipose tissues (BAT) where their stimulation lipolysis and thermogenesis respectively. BAT also plays an important role in the maintenance of glucose homeostasis. Hence beta 3 adrenergic agonists are important for treating diabetes as well as obesity.

2. **Experimental Methods**

2.1 Materials

According to the requirements of the thesis work, all chemicals were purchased partly from Research Lab, Sigma-Aldrich, SD Fine, Spectrochem and Merck companies. The yields of the products obtained, refer to purified products and are not optimized. Melting points of synthesized organic compounds were determined on VEEGO - VMP I melting point apparatus and are uncorrected. IR spectrums were recorded on JASCO-FTIR 4100 spectrophotometer and are expressed in cm-1. 1H NMR was recorded at the University of Pune in the analytical department using Mercury Varian 300 MHz. chemical shifts (δ) were reported in parts per million (ppm) with CDCl3 7.26 ppm as a solvent. TMS was used as internal standard for NMR. Chemical shifts peak were represented by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet).Mass spectrums were recorded at the University of Pune. Thin layer chromatography (TLC) was performed on aluminum plate pre-coated with Silica Gel 60 F254.

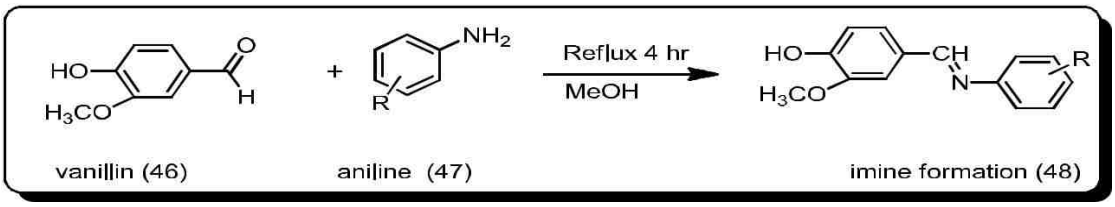
2.2 Synthesis of Aryloxypropanolamines derivatives



General scheme of synthesis

2.2.1 Synthesis of step 1 intermediate (imine intermediate)

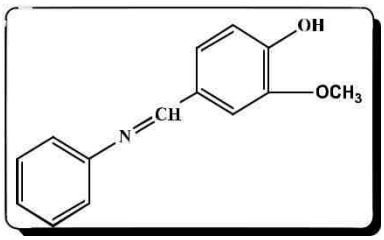
The substituted aniline (0.0329 moles, 1 equiv.) was dissolved in methanol (20 ml, 4 vol.) in a beaker and in another RBF vanillin (0.0329 moles, 1 equiv.) was dissolved in methanol (20 ml, 4 vol.) with continuous stirring. The solution of substituted aniline was slowly added drop by drop in the solution of vanillin with continuous stirring. On completion of addition, the mixture was allowed to reflux for 1-4 hours as per the requirement while with continuous monitoring on TLC at the interval of every 30 minutes. After completion of the reaction, the reaction mixture was poured into an evaporating dish and allowed the solvent to evaporate or the excess of solvent was removed by applying the vacuum at a particular pressure. The solid was isolated and a melting point of the obtained imine was determined.



2.2.1.1 Physicochemical parameters of step 1 intermediate (imine intermediate)

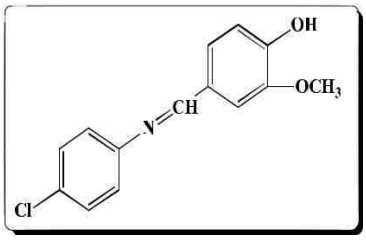
Sr. No.	Compound Code	R	Melting*/Boiling Point (°C)	% Yield
1	S_1	H	120-123	82.26
2	S_2	4 - Cl	108-125	80.84
3	S_3	3,4- dichoro	185-190	91.57
4	S_4	2 – CH_3	182-187	79.18
5	S_5	3 – OCH_3	165-170	92.61

2.2.1.2 Characterization of synthesized step1 intermediate (imine intermediate)

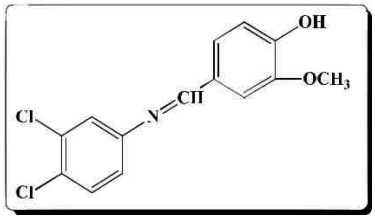


4-((phenylimino)methyl)phenol (S1): % yield : 82.26, Molecular formula :C13H11NO, Molecular weight:197.23, Melting point: 1200 to 1230, Rf : 0.63 (Hexane: Ethyl

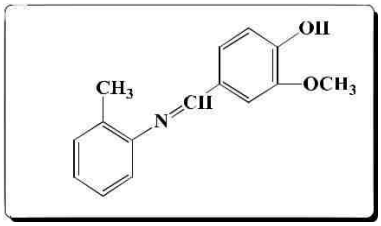
acetate,1:0.5), I.R. (KBr) cm-1:3050-3000 (aromatic hydrocarbon), 2869 (C-H stretching), 2210-2260 (C=N), 1710-1690 (Ar-CHO), 1400 (alkene C-H bend), 1180-1360 (amines);1H NMR (600 MHz, CDCl3) δ [ppm]: 7 - 7.5 (m, 5H, aromatic H), 5.5 (d, -OH), 7.52 (m,benzylidenimin).



4-(((4-chlorophenyl)imino)methyl)phenol (S2): % yield : 80.84, Molecular formula : C13H10ClNO, Molecular weight:231.68, Melting point: 108-1830C, Rf : 0.57 (Hexane: Ethyl acetate,1:0.5), I.R. (KBr) cm-1: 3050-3000 (aromatic hydrocarbon), 2869 (C-H stretching), 2210-2260 (C=N), 1400 (alkene C-H bend), 1180-1360 (amines); 1H NMR (600 MHz, CDCl3) δ [ppm]: 6.35 (s, OH), 7.5-7.8 (m, benzene), 2.34 (s, 3H, methyl).

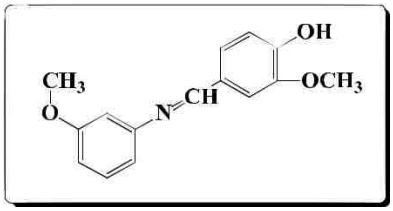


4-(((3,4-dichlorophenyl)imino)methyl) phenol (S3) %yield : 91.57, Molecular formula : C13H9Cl2NO, Molecular weight:266.12, Melting point: 185-1900C, Rf : 0.56 (Hexane: Ethyl acetate,1:0.6), I.R. (KBr) cm-1: 3439.19, 3063.06 (aromatic hydrocarbon), 2837.38 (C-H stretching), 1863.3, 1597.11, 1429.95 (alkene C-H bend), 1379.15, 1242.20 (amines), 1035.81, 976.01, 904.01, 819.77(C-Cl).



4-((m-tolylimino)methyl)phenol (S4) : %yield: 79.18, Molecular formula : C14H13NO, Molecular weight:211.26, Melting point: 182-1870C, Rf : 0.51(Hexane: Ethyl acetate,1:0.5), I.R. (KBr) cm-1: 3050-3000 (aromatic hydrocarbon), 2869 (C-H stretching), 1600-1700 (C=N

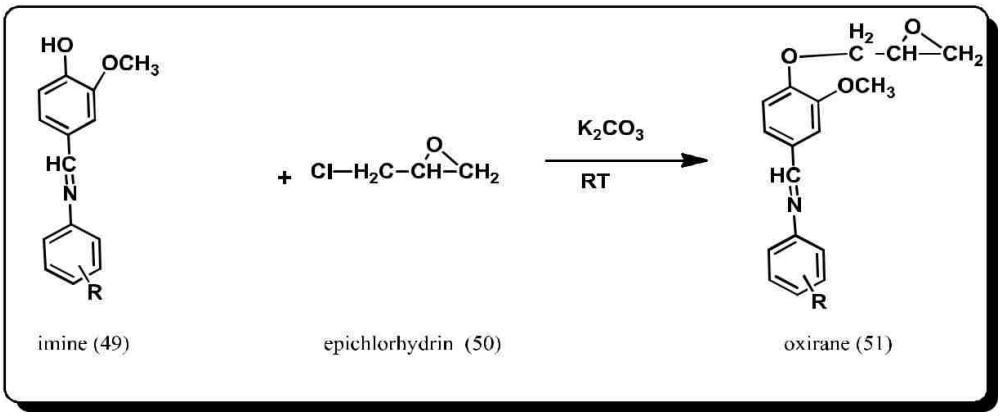
strech), 1400 (alkene C-H bend), 1300-1500 (C-H bending), 1171-1690 (Ar CHO), 1180-1360 (amines).



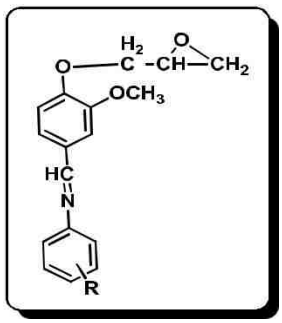
4-(((3-methoxyphenyl)imino)methyl)phenol (S5): % yield: 92.61, Molecular formula: C14H13NO2, Molecular weight:227.26, Melting point: 165-1700C, Rf: 0.54 (Hexane:Ethyl acetate,1:0.5)

2.2.2 Synthesis of step 2 intermediate (oxirane intermediates)

A mixture of the Imine (0.33 mole, 1 equivalent), Potassium carbonate) (0.66 moles, 2 equivalent) and epichlorhydrin (70ml) were heated at 750C with stirring for 10 hr, cooled to room temperature, and filtered. The filtrate was concentrated under vacuum to afford oxirane intermediate.



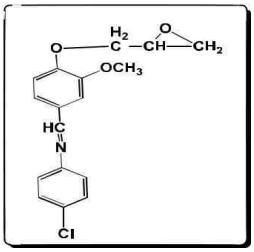
2.2.2.1 Physicochemical parameters of step 2 intermediates (oxirane intermediates)



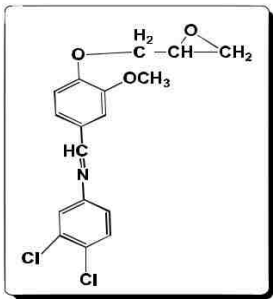
Sr. No.	Compound	R	Yield %	Melting*/Boiling

	Code			Point (°C)
1.	E1	-H	42.50	230-240
2.	E2	4-Cl	51.10	265-275
3.	E3	-3,4 dichloro	53.30	212-220
4.	E4	2-CH_3	57.69	262-270
5.	E5	3-OCH_3	60.43	235-245

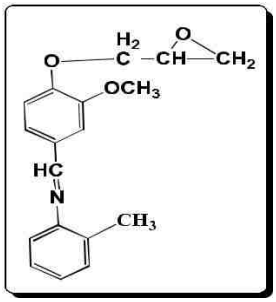
2.2.2.2 Characterization of step 2 intermediates (oxirane intermediates)



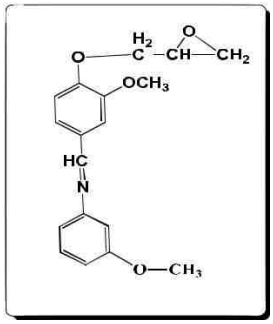
N-chloro-N-(3-methoxy-4-(oxiran-2-ylmethoxy) benzylidene)aniline (E2): %yield: 51.10, Molecular formula: C17H16ClNO3, Molecular weight: 317.77, Boiling point: 265-275 0C, Rf: 0.48 (Hexane: Ethyl acetate, 70:30).



3,4-dichloro-N-(3-methoxy-4-(oxiran-2-ylmethoxy) benzylidene)aniline(E3): % yield: 53.30, Molecular formula: C17H15Cl2NO3, Molecular weight: 352.21, Boiling point: 212-220 0C, Rf: 0.52 (Hexane: Ethyl acetate, 70:30).



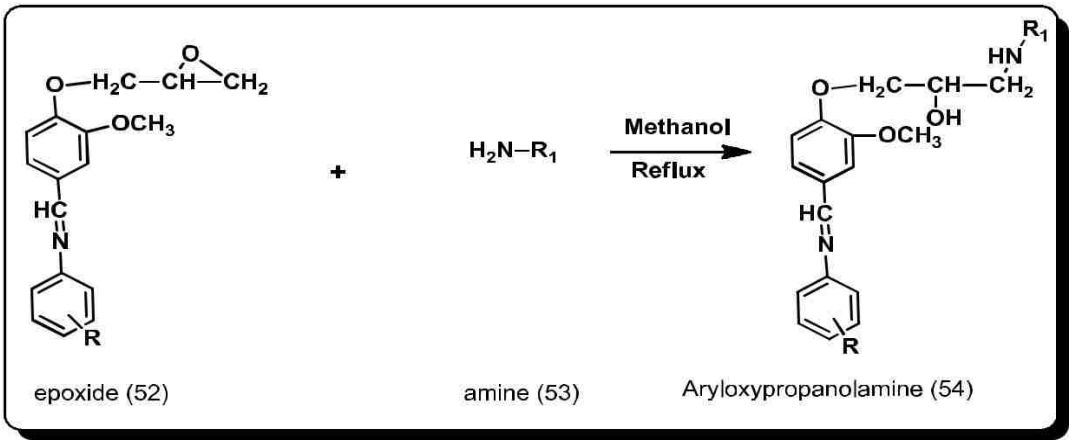
N-(3-methoxy-4-(oxiran-2-ylmethoxy) benzylidene)-2-methylaniline (E4): % yield: 57.69, Molecular formula: C18H19NO3, Molecular weight: 297.35, Boiling point: 262-2700C, Rf: 0.76 (Hexane: Ethyl acetate, 70:3



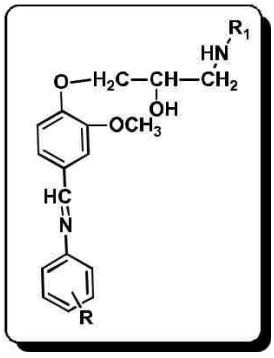
N-(3-methoxy-4-(oxiran-2-ylmethoxy) benzylidene)aniline (E1): % yield: 42.50, Molecular formula: C17H17NO3, Boiling point: 230-2400C Molecular weight: 283.32, Rf: 0.51(Hexane: Ethyl acetate, 70:30).

2.2.3 Synthesis of final step derivatives (aryloxy propanolamine derivatives)

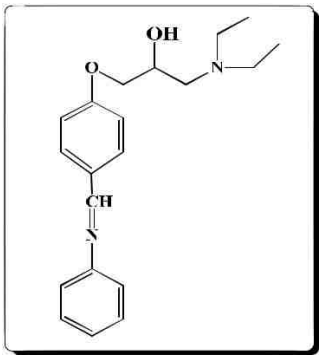
Oxirane intermediate (0.316 moles, 1 equivalent), to this add t-butylamine diethylamine (0.95 moles, 3 equivalent) and water (30ml) were combined with continuous stirring at room temperature for 12 hr. as per the requirement. Completion of the reaction was monitored by TLC. The excess of add tertiary butyl amine/diethylamine was removed by vacuum distillation. The resulting residue was extracted with ethyl acetate (2*300ml). The organic layer was dried over anhydrous sodium sulfate and concentrated to give aryloxy propanolamine. Further purification was done by column chromatography.



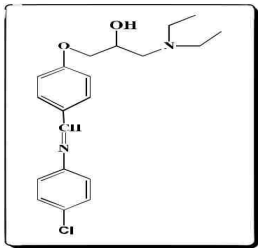
2.2.3.1 Physicochemical parameters of final step derivative (Aryloxypropanolamine derivatives)



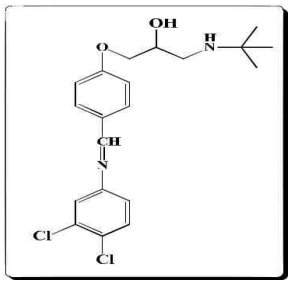
2.2.3.2 Characterization of final step derivatives (Aryloxypropanolamine derivatives)



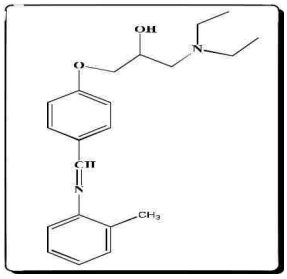
1-(isopropylamino)-3-(4-((phenylimino)methyl)phenoxy)propan-2-ol(A1):% yield: 42.5, Molecular formula: C19H24N2O2, Molecular weight: 312.41, Melting point: 112-1150C, Rf : 0.48 (Hexane: Ethyl acetate,70:30); I.R. (KBr) cm-1: 3244 (-OH), 3045 (=C-H str., Ar.), 2946-2845 (C-H Str.), 1550 (C=C str., Ar.), 1100 (C=N).



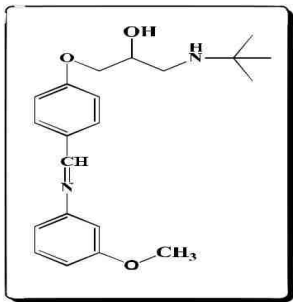
1-(tert-butylamino)-3-(4-(((4-chlorophenyl)imino)methyl)phenoxy)propan-2-ol(A2): % yield: 42.5, Molecular formula: C20H25ClN2O2, Molecular weight: 360.88, Melting point: 95-1000C, Rf : 0.48 (Hexane: Ethyl acetate,70:30), I.R. (KBr) cm-1: 3244 (-OH), 3045 (=C-H str., Ar.), 2946-2845 (C-H Str.), 1618(C=N), 1670, 1550 (C=C str., Ar.).



1-(tert-butylamino)-3-(4-(((3,4-dichlorophenyl) imino)methyl)phenoxy)propan-2-ol(A3): % yield: 30.43, Molecular formula :C20H24Cl2N2O2, Molecular weight:395.32, Melting point: 125-1300C, Rf: 0.45 (Hexane : Ethyl acetate,70:30); I.R. (KBr) cm-1: 3046 (OH), 2950 (C-H str.), 1681, 1590-1511 (C=C str., Ar.), 1618 (C=N), 750, 652 (C-Cl str.).



1-(diethylamino)-3-(4-((o-tolylimino)methyl)phenoxy)propan-2-ol (A4) : % yield:36.73, Molecular formula: C21H28N2O2, Molecular weight: 340.46, Melting point: 91-960C, Rf value: 0.46 (Hexane: Ethyl acetate, 70:30); I.R. (KBr) cm-1: 3030 (OH), 2890 (C-H Str.), 1600, 1555-1520 (C=C, Str. Ar.), 1365 (C-N str.).



1-(tert-butylamino)-3-(4-(((3-methoxyphenyl)imino)methyl)phenoxy)propan-2-ol (A5): % yield: 61.53, Molecular formula: C21H28N2O3,Molecular weight: 356.46, Melting point: 174-1790C, Rf: 0.51 (Hexane: Ethyl acetate, 70:30); I.R. (KBr) cm-1: 3056 (-OH), 1514 (aromatic ring), 1676, 2315 (-CN), 3400 (-NH), 1139 (-CN) amine.

3. Anti-diabetic activity of Aryloxypropanolamines

All the synthesized compounds (A1-5) were evaluated for their anti-diabetic activity in alloxan-induced β-cells damaged diabetic model of Wistar strain of male rats. Blood glucose levels of the glucose loaded and alloxan induced diabetic rats were estimated over 360 minutes using a glucometer, by collecting the blood from the tail of the rat. study approval/study protocol was obtained from the Institutional Animal Ethics Committee (IAEC) of P.E. Society's Modern College of Pharmacy constituted under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Approval Number is MCP/IAEC/08/2011.

3.1 Animals

Male Wistar rats (150 gm-250 gm) were taken from the Yash Farm, Aundh, Pune, India and were housed at a temperature of 250C ± 10C and relative humidity of 45% to 55% in a clean environment under 12:12 hours light/ dark cycle. Before and during the experiment the animals were fed with standard diet (Neutralight life sciences, Aundh Pune) and filtered water was available. After randomisation into various groups and before initiation of the experiment, the rats were acclimatized for a period of 7 days in standard environmental conditions and temperature as above. Animals described as fasting were deprived of food for 16 hours.

3.2 Chemicals

The pharmacological agents used were alloxan (Sigma chemicals Co., Louis, MO, USA) 120mg/kg body weight, Gliclazide 8.3mg/kg body weight, Sodium CMC (Loba Chemicals), Saline water Fresh drug solutions were prepared on the day of the experiment. The alloxan was dissolved and diluted to appropriate concentration with physiological saline (0.9% NaCl). The standard drug used Gliclazide and test drugs aryloxy propanolamine in the concentration of 8.3 mg/kg body weight was dissolved in 0.3% of Sodium CMC prepared in distilled water.

3.3 Acute toxicity study

The acute toxicity study is performed by oral administration of aryloxy propanolamine in the dose of 5mg-100mg/kg body weight of only male Wistar rats. There could be only 2 animals in each group. A wide range of doses can be tested starting from the lowest dose with increments of 2. The simple method of up and down or staircase method is followed. The two rats are injected with a particular dose of the drug and observed for the 24 h for any mortality. The subsequent doses further increased by factor 1.5 if the dose is tolerated. The dose given for trial was 1mg/kg body weight and further doses were decided.

Table 4.3: Amount of dose and animal no. of dead in LD50

Groups	Dose (mg/ kg body wt.)	No. of animals dead
I	2	0
II	3	0
	4.5	0
IV	6.75	0
V	10.125	0
VI	15.187	0
VII	22.78	0
VIII	34.17	0
IX	51.26	1
X	76.89	2

Determination of blood glucose level using alloxan induced diabetes

Thirty-six male Wistar rats were weighing about 150-250 gm were made diabetic by intraperitoneal injection of Alloxan (120mg/ kg body weight). The rats were weighed on the balance, their body weight is noted and then they were grouped as n=5 in different 6 cages. The cages were labeled properly. The dose of alloxan given was 120mg/kg body weight of each rat.

After injecting the alloxan monohydrate the animals were having free access to the food, for 48 hours. After completing 48 hours the rats were kept for fasting for another 16 hours and their fasting blood glucose level is monitored, by using Accu-chek Sensor Comfort glucometer strips on Roche glucometer". The rats with blood glucose level above 200mg/dl and signs of polyuria,

polydipsia, polyphagia, weight loss, glucosuria, and hyperglycemia were selected for the experiment.

Table 5.4: Decrease in blood glucose level after administering the test drug and the standard drug with increasing hours:

Sr. No.	Groups of animals	Blood glucose levels at specific time intervals (mg/dl) and its average values are given (n=5)							
		0h	1/2h	1h	2h	3h	4h	5h	6h
1	Normal control	135	131	124	168	120	192	172	149
2	Diabetic control	321	332	347	356	363	370	391	393
3	Std. Drug	282	278	253	229	218	200	187	171
4	Test drug: A1	301	277	265	244	231	222	203	183
5	Test drug: A2	298	256	238	226	212	201	189	176
6	Test drug: A3	289	244	240	233	230	220	209	195
7	Test drug: A4	310	300	285	266	250	234	222	212
8	Test drug: A5	304	253	231	215	210	200	189	170

The Standard dose of Gliclazide given was 8.3mg/kg body weight.

The Test drug dose given was 42.715 mg/ kg body weight.

4. Conclusion

An efficient route of synthesis of Aryloxypropanolamine derivatives was carried out and they are subjected to evaluation of anti-diabetic activity using alloxan induced diabetes mellitus. The synthesized derivatives (A1 to 5) and all the intermediates are confirmed by spectroscopic methods like NMR, IR and Mass spectrum. The compound A5 having OCH3 group has shown highest activity. It has shown 35.60% and 57.52 % decreases in blood glucose level after 2 hr and 6 hr respectively. The compound A5 which has shown good activity as mentioned above consist of t-butylamine on oxypropylamine side chain, but the compound A3 consisting of similar t-butylamine group and disubstituted aniline has shown less activity. The compound A2 consisting of electron donating chloro group on Meta position and diethylamine has shown good activity. Compound A1 and A3 consisting of aniline-isopropylamine and ortho-toluidine-isopropylamine respectively have shown moderate antidiabetic activity.

References

- Main B G, Tucher H, Medicinal Chemistry: The Role of Organic chemistry in Drug Research of β blockers, edited by Roberts S M and Price B J, (Academic Press, London), 1985.
- Howe R. Crowther A. F. Stephenson J. S. Rao B. S, Smith L. H.β-Adrenergic blocking agents. I. Pronethalol and related N-alkyl and N-aralkyl derivatives of 2-amino-1-(2-naphthyl)ethanol. J Med Chem. 1968; 11:1000- 1008.
- Crowther A , Smith L, β -Adrenergic blocking agents. II. Propranolol and related 3-amino-1-naphthoxy-2-propanols. J Med Chem. 1968;11:1009-1013
- Howe R, Rao B.S. β -Adrenergic blocking agents. III. The optical isomers of pronethalol, propranolol, and several related compounds. J Med Chem. 1968;11;1118-1121.
- Powell J, Wainer I, Drayer D, Drug stereochemistry Analytical Methods and Pharmacology, (Marcel Dekker, USA), 1988.
- Gas D, Kregar M. Am Inter Med. 1970;70:985.
- Somerville. A, Coleman A, Patersom D, Biochem. Pharmacol.; 1979;28;1011.
- Frykman H, Ohner N, Martinille M, Mattson A, Norin T, Hult K, Biocatalysis;1994; 9; 105.
- Kubo A, Aoki H, Nanba H, Anti-diabetic activity present in the fruit body of Groola frondosa (Maitake). Biological & pharmaceutical bulletin; 1994;17(8);1106-1110.

LIST OF GRAPHS

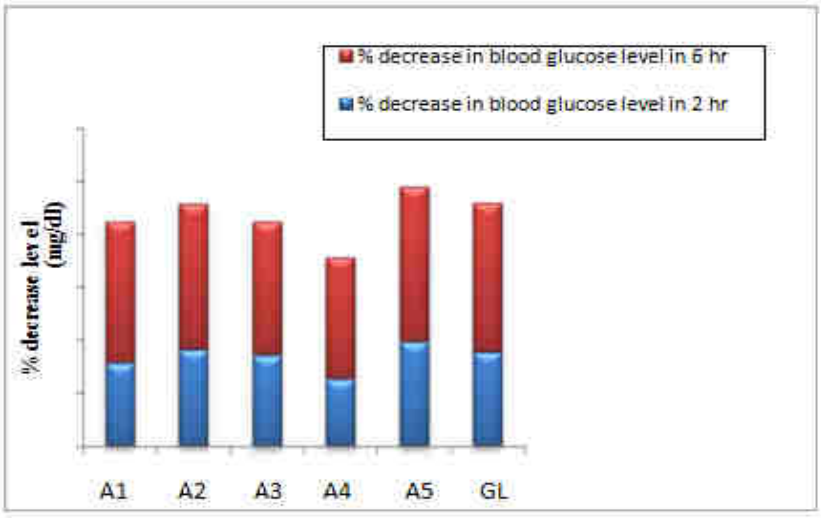


Figure 5.1: Graphical representation of blood glucose level of Normal control