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Research Article

SYNTHESIS OF 4-(BENZYLAMINO)-6-CHLORO-2, 2-DIMETHYL-3, 4-DIHYDRO-2H-CHROMEN-3-OL FOR ANTIHYPERTENSIVE ACTIVITY

***M. Bano and S.M. Ahmed**
Department of Pharmaceutical Chemistry,
Krupanidhi College of Pharmacy Carmelaram Post,
Chikkabellandur , Bangalore-560035. India.
**Author for Correspondence*

ABSTRACT
The synthesis of 4-(benzylamino)-6-chloro-2, 2-dimethyl-3, 4-dihydro-2H-chromen-3-ol was achieved. These compounds were synthesized from different substituted phenols as starting material. The phenols acetylated in the first step and undergone Fries rearrangement in the second step to afford ortho hydroxyl acetophenone moiety **3a-e**. These further treated with acetone in toluene in the presence of piperidine to afford benzopyaranone**4a-e.** The compound **4a-e** was further reduced by sodium boro hydrate in methanol to obtain 4-hydroxy benzopyran **5a-e.** The compound **5a-e** was refluxed under Dean Stack condition using p-toluene sulphonic acid to afford benzopyrans**6a-e**. These compounds are converted to epoxide **7a-e** by treating with sodium hyphochloride in the presence of phosphate buffer and DCM- water as solvent. The final step is the ring opening reaction of the epoxide by substituted benzylamines in the presence of magnesium sulphate. All the target compounds 8a-j werecharecterised by IR, NMR and Mass spectral data and tested for in vivo antihypertensive activity.

Key Words: *Benzopyrans, Cromakalim, Epoxide, Potassium Channels Openers, Antihypertensive Activity, Cardioprotective Agents*

INTRODUCTION
There are large number of compounds in the market for reducing hypertension. They work with different mechanism and no compound is found to be successful in completely curing the hypertension. Some compounds are also known to produce undesirable toxicity. The increase in the number of hypertensive patient in developing countries in very rapid rate and if not treated it will lead to various cardiovascular disorders. One of the newly accepted mechanism in opening potassium channel for the treating hypertension are related to ATP sensitive potassium channel openers. There are number of compounds works with this mechanism in preclinical and clinical stage and the examples include Cromakalim and BMS compounds. Potassium channels openers are a class of compounds which possess a wide variety of pharmacological properties, they protect ischemic myocardium which is independent of vasodilator activities and effects on action potential. Benzopyran derivatives substituted with secondary amines including imidazole have been pharmacologically useful in the protection of heart and neuronal cells against ischemia reperfusion injury. These channels have been found and characterized at the cellular level, they regulate changes of Adenosine Triphosphate at the intracellular lelvel (Tyrell *et al.,* 2008). They play a complex role in the basic electrical and mechanical function of wide variety of tissue including smooth muscle and glands (Richardson *et al.,* 2007). Cromakalim, a benzopyran is provided with a specific affinity towards these channels (Norman *et al.,* 2006 and Gopalkrishnan *et al.,* 2004). They have direct cardioprotective properties independent of their vasodilator action (Manley *et al.,* 2007, Breschi *et al.,* 2006). Intensive research on the development of above said pharmacological efficacies by the inventors found that the benzopyran derivatives substituted with secondary amines including imidazole exhibit various pharmacologic activities like suppression of angiogenesis, invivo anticancer activity, Cox-2 inhibitory activities. Many of the compounds related to K_{ATP} 4-(N-Imidazol-2-ylmethyl) amino Benzopyran have also shown inhibitory effects on HUVEC(human umbilical vein endothelial cell) tube formation indicating antiangiogenic properties (Lee *et al.,* 2000). Imidazole analogue of 4-(N-aryl)-substituted benzopyran (BMS-191095) has also been reported as acardioselective to K_{ATP} Opener (Cho *et*

al., 2008, Norman *et al.,* 1994). Hence an attempt has been made to synthesize and explore the pharmacophoric activities of this heterocyclic compound with the Benzopyran which is known to possess wide variety of pharmacological activities. Hence we thought to design and synthesize a similar type of compound and test for antihypertensive activity.

RESULTS AND DISCUSSIONS

The scheme of synthesis for the target benzopyran analogue is depicted in the scheme-1. We selected different substituted phenols as starting material for the synthesis of target compounds. Here we used chloro phenol, bromophenol, benzoic acid, amino phenol andnitrophenol as starting material. These phenols were acetylated by treatment with acetyl chloride in the presence of triethylamine as a base and dichoromethane as solvent. This reaction was tried in roomtemperature and at 0^oC. However reaction at 0^oC is found be of better yield and smooth reaction. In the next step O-acetylated product undergoes Fries type of rearrangement in the presence of aluminium chloride to obtain the orthohydroxyacetophenone moiety **3a-e**. The temperature for the reaction very important and at 140-150^oC the reaction is taking place and at higher temperature reaction become charred. Hence it is very important to main the temperature and we used sand bath for maintaining the exact temperature. The compound 3a-e was conformed initially by melting point and then by IR spectral data. The presence of the of broad peak at 3400cm-1 indicated the presence of OH stretching vibration and the shift of carbonyl peak of O-acetylated compound and ortho hydroxyl moiety confirms the structure of the compound. In the next step, theseorthohydroxy moiety is converted to benzopyranone by treatment with acetone under refluxing conditions in the presence of piperidine as catalyst and toluene as solvent to obtain the compound 4a-e. The formation of the compound is confirmed by IR and NMR spectral data. Here in the IR we observed the absence of broad peak at 3400 for the hydroxyl group and shift of the carbonyl stretching vibration from 1740 to 1710 indicated the formation of the ring. In the NMR spectra we observed that there is absence of acetyl group at 2.8. Further these compound 4a-e were reduced to hydroxyl function by treating with sodium brohydrate in methanol. Sodium borohydrate is a good reducing agent and the reaction has taken place very smoothly in room temperature forming a single compound 5a-e. The compound 5a-e was confirmed by IR spectroscopy here we observed that the presence of broad peak at 3430 indicating the formation of hydroxyl compound. There is also absence of carbonyl stretching vibration at 1710 which conforms the formation of 5a-e.

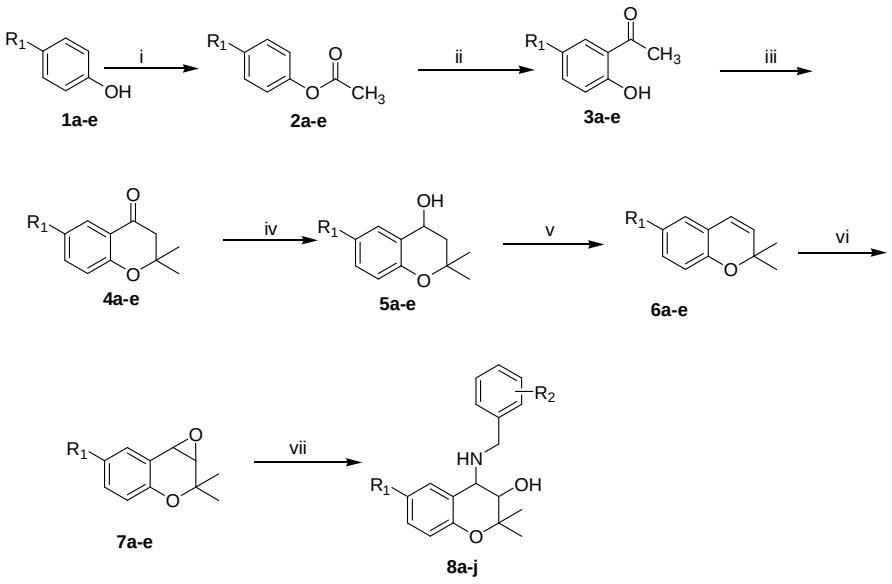
We have converted the 4-hydroxy benzopyran moiety to benzopyran in the next step by refluxing with toluene in the presence of paratoluenesulphonic acid as catalyst. The reaction requires higher temperature and we used Dean Stack apparatus for removal of water molecules in the reaction. The reaction took 12-18 hrs under refluxing condition for complete formation of the 6a-e. The compound was confirmed by IR spectral data. Here we observed the absence of hydroxyl group at 3430 indicated the formation of 6a-e. In the next step we prepared different epoxides by treating with sodium hypochloride in the presence of phosphate buffer. The phosphate buffer is very important for maintaining PH of the solution. The combination of water and dichlormethane is used as solvent. Because the all the inorganic compounds are not soluble in DCM and it should be dissolved in water before starting the reaction. Minimum amount of water is used and then added DCM and reaction carried at room temperature. The formation of 7a-e is confirmed by IR and NMR spectral data.

In the last step these compounds were treated with different substituted benzylamine to form final target compound 8a-j. This is the ring opening reaction and we find by use of different benzylamines ring opening is taking place and we are able to do the reaction in magnesium sulphate smoothly in the presence of DCM as solvent. The compound 8a-j were confirmed by IR, NMR and Mass spectral data.

Experimental

Melting points are taken in open capillary tubes and are uncorrected. Precoated TLC plates are purchased from Sigma alrich are used directly. IR spectra are taken in Perkin Elmer FTIR spectrophotometer and NMR in Bruker 300MHZ Instrument.

Scheme-1: Synthesis of Benzopyran derivatives.



R1= Cl, Br,COOH NH2, NO2
R2= H, 4-CH3

Reagents and Conditions: i) CH3COCl, DCM, 0^{0}C, ii) AlCl3, 140-150^{0}C, iii) CH3COCH3, Pipperidine, reflux iv) NaBH4, MeOH, rt v) PTSA, Toluene, reflux vi) NaOCl, NaHPO4, DCM-H2O vii) MgCl2, DCM reflux

Synthesis of 4-Chlorophenyl Acetate 2a-e:

One equivalent of parachlorophenol was dissolved in dichloromethane in a round bottomed flask, to that two equivalent triethylamine was added drop-wise and kept in ice cold temperature of 0-5^{0}C. Two equivalents of acetyl chloride was added drop-wise and kept in ice cold temperature of 0-5^{0}C with constant stirring to the above mixture. After about 3-4hrs the reaction mixture was kept at room temperature for 3 hrs, TLC was carried out to confirm the completion of reaction, crushed ice was added and extracted with dichloromethane and DCM layers were dried with anhydrous sodium sulphate to obtain the product.

Synthesis of 1-(5-Chloro-2-Hydroxyphenyl)Methadone 3a-e:

Anhydrous aluminum chloride was added to the above product and was heated carefully at 120-140^{0}C for 12 hrs by keeping $Cacl_2$ guard tube, with constant stirring. DiluteHcl was added to get the compound. The solid was then weighed.

Synthesis of 6-Chloro-2, 2-Dimethyl-2, 3-Dihydrochromen-4-One 4a-e:

To the above intermediate added acetone, pyrrolidine in an Round Bottomed Flask with a Dean Stack apparatus fixed with toluene up to the mark, refluxed for 4hrs, after 4 hrs TLC was carried out to check the completion of the reaction, reflux time was increased to about 12 hrs to modify the yield, further the solvent was removed by distillation. It was further extracted using water and ethyl acetate and EC layers were dried using anhydrous sodium sulphate to obtain product

Synthesis of 6-Chloro-2, 2-Dimethyl-3, 4-Dihydro-2H-Chromen-4-ol 5a-e:

The above intermediate in 100ml methanol was cooled and added sodium borohydratedropwise, kept at room temperature, it was left overnight for better yield. TLC is performed to check the completion of the reaction, finally the reaction mixture was quenched with water to obtain product and the yield was found to be 45%.

Synthesis of 6-Chloro-2, 2-Dimethyl-2H-Chromene 6a-e:

One milli mole of the compound in toluene was added with 0.03mmol of p-toluene sulphonic acid and refluxed for 3 hrs, after 3 hrs the reaction mixture was checked for the completion using TLC. Aqueous

NaHCO3 was added and extracted with ethyl acetate, crude was treated with anhydrous MgSO4 and recrystallized with ethyl acetate. The yield was found to be 50%.

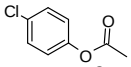
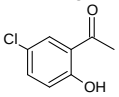
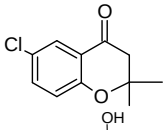
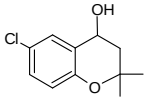
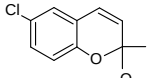
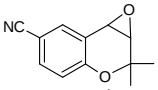
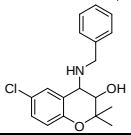
Synthesis of 6-Chloro-2, 2-Dimethyl-2, 7b-Dihydro-1aH-Oxireno(2, 3-c)Chromene 7a-e:

Sodium hypochlorite and sodium phosphate are taken in an conical flask, added 5ml of water and cooled to0^0C. To the above mixture 0.5 gm of Chloro Benzopyran dissolved in Methylene chloride, cooled to 0 was added dropwise using a dropping funnel maintaining the temperature of0^0C throughout the reaction, After about 3hrs, further 1 equivalent of Sodium hypochlorite was added to the reaction mixture. After about 20hrs reaction was stopped, reaction mixture was filtered with celite and was extracted with Methylene chloride. Crude mixture was finally purified by using Column Chromatography. The reaction was confirmed by TLC, IR and NMR.

Synthesis of 4-(Benzylamino)-6-Chloro-2, 2-Dimethyl-3, 4-Dihydro-2H-Chromen-3-ol 8a-j:

Equimolar quantity of benzylamine and compound 7a is taken in a round bottom flask and 2 moles of magnesium sulphate is added in methanol. The reaction mixture was stirred at room temperature for about 6 hrs. Reaction was monitored by TLC and after completion of the reaction it was filtered and the filtrate is concentrated and added to water. The residue obtained was purified by column chromatography and characterized by IR NMR and Mass spectral data. (Table 1 and 2)

Table 1: Analytical data of Synthesized compounds 2a-8a

Comd No	STRUCTURE	MOL.FORMULA	M.P (^{0}C)	Rf VALUE	% YIELD
2a		**$C_8H_7O_2Cl$**	*155*	*0.7*	*70*
3a		**$C_8H_7O_2Cl$**	*145*	*0.6*	*60*
4a		**$C_{11}H_{11}O_2Cl$**	*175*	*0.2*	*45*
5a		**$C_{11}H_{13}O_2Cl$**	*160*	*0.5*	*35*
6a		**$C_{11}H_{11}OCl$**	*192*	*0.4*	*40*
7a		**$C_{11}H_{11}O_2Cl$**	182	0.6	50
8a		**$C_{18}H_{20}ClNO_2$**	178	0.5	62

Antihypertensive Activity

Six groups of animals each containing 3 animals were initially selected, At first as per the Guidelines No. 420 and 421, given a dose of 700mg/kg body weight, monitored the animal for the toxic symptoms as well as mortality, the animals showed high toxicity symptoms such as increased intestinal motility, diarrhea, tail erection, irritation to nose etc., and all the animals were Dead. Hence we decreased the dose to 500 mg/kg body weight and administered to the next group of animals, monitored for toxic symptoms and mortality. In this dose animals were safe but showed fewer toxic symptoms and only few were mortal, toxicity symptoms were diarrhea, tail erection and irritation to the nose. Once again we choose a

dose of 300mg/kg body weight to the next set of animals and observed for the toxic symptoms and mortality.

Table 2: IR and NMR spectral data of synthesized compounds 8a-e

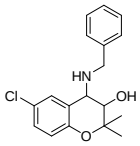
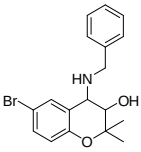
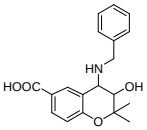
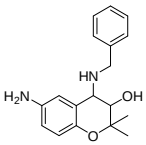
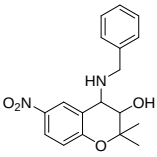
S. No.	Compd No	Structure	IR DATA(cm^{-1})	NMR DATA (δ ppm, 300MHz DMSO-d_6)
1	8a		1149.614(ARClst), 1203.62(C-O-C st), 1620.26(C=C st), 3057.27(CH Arst), 2978(CH Ali st), 1194(C-C st).	2.193(s,6H,CH3),7.267(s,1H,Ar H),.5.710(d, 1H CH), 6.925(d,1H,CH),7.3833(d,1H,ACH) , 6.806 (d, 1H, ArH)
2	8b		333.89(OHst),1263.42(C-O-st),2958.90 (CH Ali st), 3057.27(C-H Arst), 1668(C=C st), 1170(Ar-Clst)	2.8(s,6H ,CH3), 7.9(s,1H Ar H), 6.783(d,1H,ArH),7.362(d1H,ArH), 2.2(s,2H,CH), 6.783(d 1H ArH)
3	8c		1120(Ar-Clst), 1716.70(C=O st), 914.29 (C-C st), 1259(C-O-C st), 2989.76(C-H Ali st), 3103.57(C-H Ar- st),	2.8(s,6H ,CH3), 7.9(s,1H Ar H), 6.783(d, 1H, ArH) 7.362(d1H,ArH), 2.2(s,2H,CH), 6.783(d 1H ArH)
4	8d		2226.05(CNst), 1213(C-O-C st), 1602.9(C=C st), 3047.80(CH Arst), 2978.36.(CH Ali st), 1128.46(C-C st),1278,896(C-O)	3.62(d,1H,ArH)1.45, (s, 6H CH3), 6.783(d,1H ArH), 6.273(d,1H ArH), 5.694(d,1H CH), 7.243(d,1H ArH),
5	8e		2226.05(CNst), 1213(C-O-C st), 1602.9(C=C st), 3047.80(CH Arst), 2978.36.(CH Ali st), 1128.46(C-C st),1278,896(C-O)	2.8(s,6H ,CH3), 7.9(s,1H Ar H), 6.783(d, 1H, ArH) 7.362(d1H,ArH), 2.2(s,2H,CH), 6.783(d 1H ArH)

Table 3: Antihypertensive activity

Sr. No.	Compd. No	Baseline	Adrenaline	Test Alone	Test +Adrenaline
1	8a	116.4±0.4826	174.6±1.198	118.6±1.389	141.9±1.406
2	8b	57.88±0.9274	85.13±3.054	67.06±0.1073	110.3±15.41
3	8c	82.97±0.8903	142.1±1.883	93.44±0.4887	119.2±0.9313
4	8e	279.9±3.106	246.0±54.31	241.0±0.5571	232.4±3.658
5	8f	82.97±0.8903	142.1±1.883	93.44±0.4887	119.2±0.9313
6	Std	118±0.8903	95.13±3.054	83.44±0.4887	139.2±0.9313

In this dose no toxic symptoms were seen and all the animals were safe. Hence we concluded that 300mg/kg body weight dose was safe and recommended dose for further studies (antihypertensive activity).

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