ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis of 6-chloro-4-(furan-2-ylmethyleneamino)-2, 2-dimethyl-2H-chromen-3-ol for Antihypertensive Activity

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ABSTRACT

The synthesis of 6-chloro-4-(furan-2-ylmethyleneamino)-2,2-dimethyl-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 borohydrate 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 were converted to epoxide **7a-e** by treating with sodium hyphochloride in the presence of phosphate buffer and DCM- water as solvent. In the next step the ring opening reaction is done by treating with ammonia and methanol at 50-60^{o}C to afford **8a-e**. The amino alcohol functional group is treated with different substituted furfuraldehyde to afford the target compound **9a-j**. All the target compounds **9a-j** were characterized by IR, NMR and Mass spectral data and tested for in vivo antihypertensive activity.

Keywords: Benzopyrans, cromakalim, potassium channel openers, antihypertensive activity.

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ISSN: 0975-8585

INTRODUCTION

Hypertension is chronic medical condition and it is a global issue which is affecting a vast majority of population. Nearly one billion of global adult population is affected with hypertension in both developed and developing world [1]. There is large number of compounds available in market reducing the hypertension and its associated complications. They work with different mechanism and not a single compound is found to be ideal in completely curing the hypertension and its related complications. The drugs presently available in the market for the treatment of hypertension are not completely ideal as some of the available compounds are known to produce toxicity and other side effects. Number of hypertensive patient in developing countries is increasing at very rapid rate, if the issue of hypertension is not addressed promptly by employing changes in life style and with the use safe and effective drugs it will lead to various cardiovascular disorders and increase in the financial expenditure of developing countries. One of newly accepted mechanism for treatment of hypertension is by the mechanism of opening potassium channels. They are called as 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 level.[2] They play a complex role in the basic electrical and mechanical function of wide variety of tissue including smooth muscle and glands.[3] Cromakalim, a benzopyran is provided with a specific affinity towards these channels.[4],[5] They have direct cardioprotective properties independent of their vasodilator action.[6],[7] Benzopyran based cromakalims which are KATP channel openers have been reported to be a potential class of therapeutic agents possessing wide variety of cardioprotective activities such as hypertension, ischemia and angina pectoris. One of the earlier synthesized compound KC-399, a Benzopyran K+ channel opener is claimed to be possessing long duration of antihypertensive action and less tachycardia.[8] 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,[9] Cox-2 inhibitory activities. Many of the compounds related to KATP 4-(N-Imidazol-2-ylmethyl) amino benzopyran have also shown inhibitory effects on HUVEC(human umbilical vein endothelial cell) tube formation indicating antiangiogenic properties.[10] Imidazole analogue of 4-(N-aryl)-substituted benzopyran (BMS-191095) has also been reported as a cardioselective to KATP Opener.[11] Literature shows that a vast majority of substituted benzopyrans have been synthesized and evaluated clinically for potential antihypertensive activity. Hence an attempt has been made to synthesize and explore different substituted benzopyran and evaluate for potential antihypertensive activity.

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MATERIALS AND METHODS

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, nitrophenol 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 room temperature and at 0^{o}C. However reaction at 0^{o}C is found be of better yield and the progressed smoothly. In the next step O-acetylated product undergoes Fries type of rearrangement in the presence of aluminium chloride to obtain the orthohydroxy acetophenone moiety **3a-e**. The temperature for the reaction is very important and at 140-150^{o}C the reaction is taking place, as the temperature increases the reaction gets charred. We used sand for maintaining exact reaction 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, these ortho hydroxy moieties were 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 compounds 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 paratoluene sulphonic 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 compounds were 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 dichloromethane (DCM) is used as solvent. Because 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 was carried out at room temperature. The formation of **7a-e** is confirmed by IR and NMR spectral data.

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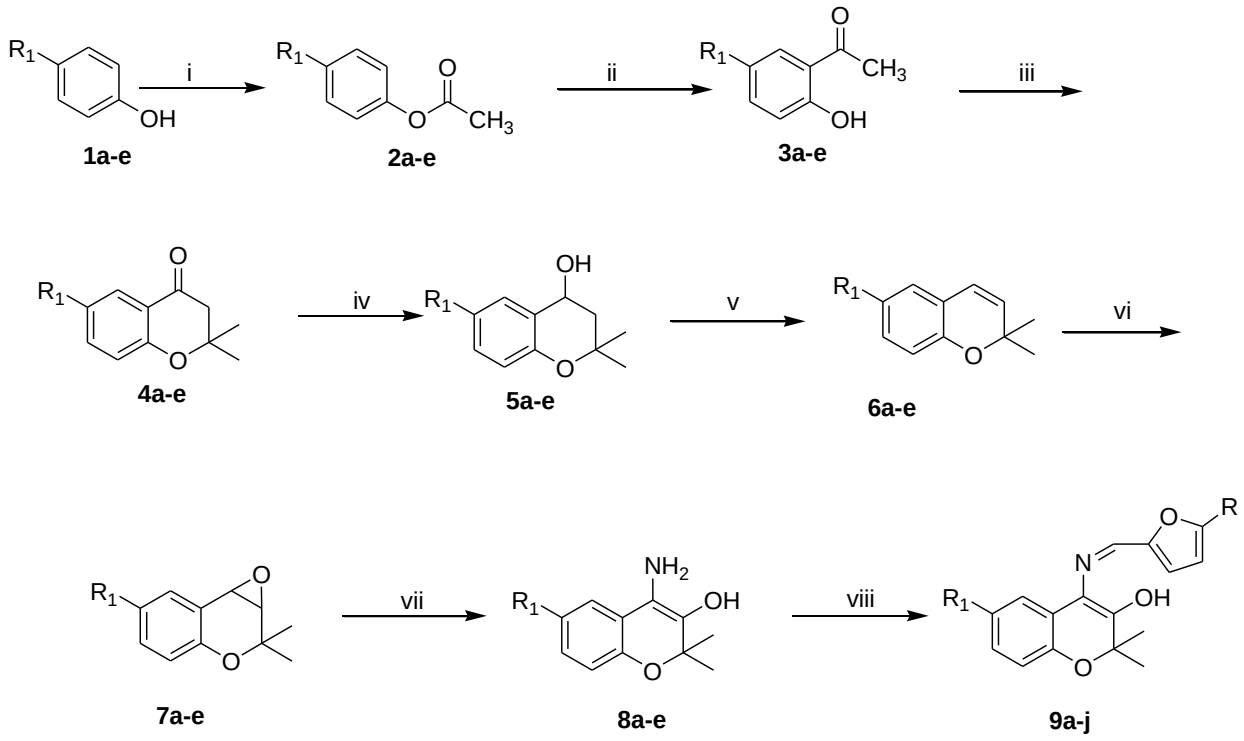
In the last step ring opening reaction for these benzopyrans were done by treating with methanol and ammonia at 50-60^{o}C to afford **8a-e**. These amino alcohol functional group of the benzopyran derivative is treated with furfuraldehyde in methanol at refluxing condition to obtain furan derivative of benzopyran **9a-j.** The compounds 9a-j were confirmed by IR, NMR and Mass spectral data.

The sequence of the reactions is depicted in the **scheme-1**.

Scheme-1: Synthesis of Benzopyran derivatives.



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

Reagents and Conditions: i) CH3COCl, DCM, 0^{o}C, ii) AlCl3, 140-150^{o}C, iii) CH3COCH3, Pipperidine, reflux iv) NaBH4, MeOH, rt v) PTSA, Toluene, reflux vi) NaOCl, NaHPO4, DCM-H2O vii) NH4OH, MeOH, 50-60^{o}C viii) Furfuraldehyde, Methanol, Reflux

EXPERIMENTAL

Melting points are taken in open capillary tubes and are uncorrected. Pre-coated TLC plates are purchased from Sigma Aldrich are used directly. IR spectra were recorded by using Perkin Elmer FTIR spectrophotometer and NMR in Bruker 300MHZ Instrument.

1. Synthesis of 4-chlorophenyl acetate 2a-e

One equivalent of parachloro phenol was dissolved in dichloromethane in a round bottomed flask, to that two equivalent triethylamine was added dropwise and kept in ice cold temperature of 0-5^{o}C. Two equivalents of acetyl chloride was added drop-wise and kept in ice cold temperature of 0-5^{o}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.

2. Synthesis of 1-(5-chloro-2-hydroxyphenyl)ethanone 3a-e

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

3. Synthesis of 6-chloro-2,2-dimethyl-2,3-dihydrochromen-4-one 4a-e

To the above intermediate added acetone, pyrolidine in an round bottomed flask with a Dean Stack apparatus fixed with toluene up to the mark, refluxed for 4 hrs, 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.

4. 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 borohydrate dropwise, 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%.

5. 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%.

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6. Synthesis of 6-chloro-2,2-dimethyl-2,7b-dihydro-1aH-oxireno[2,3-c]chromene7a-e

Sodium hypochlorite and sodium phosphate are taken in a conical flask, added 5ml of water and cooled to 0°C. To the above mixture 0.5 gm of chlorobenzopyran dissolved in methylene chloride, cooled to 0°C was added dropwise using a dropping funnel maintaining the temperature at 0°C 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.

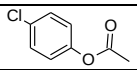
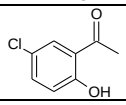
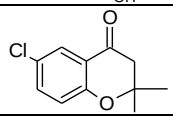
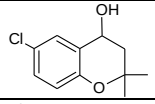
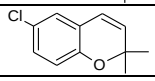
8. Synthesis of 4-amino-6-chloro-2,2-dimethyl-2H-chromen-3-ol 8a-e

The compound 7a is taken in a round bottom flask and 2 moles of ammonia and 20ml of methanol. The reaction mixture was kept at 50-60°C for about 6 hours. After completion of the reaction the reaction mixture was kept overnight. Next day the reaction mixture is added to ice water, filtered and dried to obtain the compound 8a in pure form which is used directly for the next step.

9. Synthesis of 6-chloro-4-(furan-2-ylmethyleneamino)-2,2-dimethyl-2H-chromen-3-ol 9a-e

Equimolar quantity of compound 8a and furfuraldehyde is refluxed in methanol in the presence of 2 drops concentrated sulphuric acid for about 8 hours. After completion of the reaction the reaction mixture is cooled and concentrated. The solution is added to ice water and residue obtained is filtered and dried. The residue obtained was purified by column chromatography and characterized by IR NMR and Mass spectral data. **Table-1 and Table-2**

Table-1: Analytical data of Synthesized compounds 2a-9a

Compd No	STRUCTURE	MOL.FORMULA	M.P (°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*

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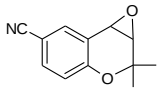
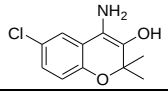
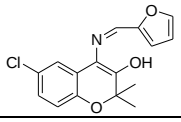
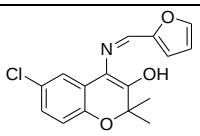
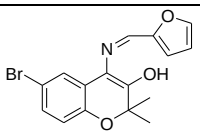
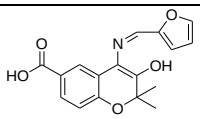
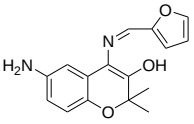
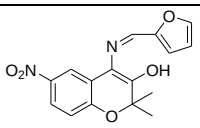
7a		**$C_{11}H_{11}O_2Cl$**	182	0.6	50
8a		**$C_{11}H_{12}ClNO_2$**	158	0.6	82
9a		**C16H14ClNO3**	144	0.5	`67

Table-2: IR and NMR spectral data of synthesized compounds 9a-e

Sl. No	Compd No	Structure	IR DATA (cm^{-1})	NMR DATA (δ ppm, 300MHz DMSO-d_6)
1	9a		3430 (OH) 1550 (C=C) 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).	2.8 (S, 1H, OH) 5.6 (S , 1H , =CH) 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	9b		3436 (OH) 1554 (C=C) 1263.42(C-O-st),2958.90 (CH Ali st), 3057.27(C-H Arst), 1668(C=C st), 1170(Ar-Clst)	2.4 (S, 1H, OH) 5.6 (S , 1H , =CH) 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	9c		3400 (OH) 1550 (C=C) 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),	2.3 (S, 1H, OH) 5.6 (S , 1H , =CH) 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	9d		3432 (OH) 1558 (C=C) 2226.05(CN st), 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)	3.8 (S, 1H, OH) 5.6 (S , 1H , =CH) 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	9e		3437 (OH) 1553 (C=C) 2226.05(CN st), 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)	3.4 (S, 1H, OH) 5.6 (S , 1H , =CH) 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)

ISSN: 0975-8585

ANTIHYPERTENCIVE 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

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

Table: Antihypertensive activity.

Sl.No	Compd.No	BASELINE	ADRENALINE	TEST ALONE	TEST +ADRENALINE
1	9a	106.4±0.4826	114.6±1.198	118.6±1.389	91.9±1.406
2	9b	87.88±0.9274	95.13±3.054	97.06±0.1073	90.3±15.41
3	9c	62.97±0.8903	92.1±1.883	93.44±0.4887	89.2±0.9313
4	9d	79.9±3.106	86.0±54.31	111.0±0.5571	92.4±3.658
5	9e	52.97±0.8903	72.1±1.883	93.44±0.4887	99.2±0.9313
6	Std	118±0.8903	95.13±3.054	83.44±0.4887	139.2±0.9313

RESULTS AND DISCUSSION

For the synthesis of desired substituted benzopyrans, substituted phenols served as suitable starting material and the procedure used was developed in our laboratory by trial and error with the help of available literature for synthesis of benzopyrans, this resulted in development of a novel procedure. Substituted benzopyran derivatives have been evaluated by several researchers internationally under different universities and pharmaceutical companies for their antihypertensive properties. Investigation of antihypertensive activity of our synthesized compounds was carried out on animals. Results of antihypertensive activity revealed that substituted benzopyrans has antihypertensive properties some compound with good antihypertensive activity and others with moderate or no activity.

CONCLUSIONS

Substituted benzopyrans are proven to have antihypertensive activity. Further research work is needed on this chemical class of compounds which may result in development of more effective, potent, long acting compounds with fewer side effects and economically cost

effective compare to compounds available in market presently for treatment of hypertension and its related complications and help in solving the global issue of hypertension.

ACKNOWLEDGEMENTS

We are thankful to Dr. Premkumar, Dean Krupanidhi College of Pharmacy and Dr. Basheeruddin , Principal Krupanidhi College of Pharmacy and Dr. Suresh Nagpal Chairman Krupanidhi Trust, for their support. I am also thankful to DST, Government of India for Woman Scientist Fellowship.

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