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Synthesis, Chemical Stability and Comparative Anti-Hypertensive Activity of Indolizine Derivatives of Propranolol

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Abstract

Prodrugs are chemically modified versions of a pharmacologically active agent, which undergo transformation in vivo to release the active drug. Prodrugs of Propranolol have been designed with an aim to achieve improved therapeutic and biopharmaceutical properties and also to reduce the extent of first pass metabolism. All the synthesized derivatives 9a-cwere subjected to drug release study and further evaluated for in vivo anti-hypertensive activity using fructose induced noninvasive tail cuff method. Among all the synthesized derivatives, 9chas shown significant anti-hypertensive activity and prolonged duration of action when compared to the standard drug Propranolol.

Keywords: Prodrugs, Propranolol, Anti-hypertensive, Non-invasive tail cuff method.

Introduction

A prodrug is a pharmacologically inactive chemical derivative that can be utilized to temporarily alter the physicochemical properties of a specific drug to increase its usefulness and minimize its toxicity. The term prodrug is usually applied to compounds that are inactive in their parent form (s) but which, after administration, are chemically transformed to the active derivative[1]. Propranolol, (Figure-1) a nonspecific β adrenergic antagonist used for the treatment of cardiac arrhythmias, angina pectoris and hypertension, undergoes extensive presystemic metabolism[2] by both glucouronide conjugation at β-OH position and aromatic hydroxylation after oral administration leading to reduced bioavailability. In order to reduce the extent of first pass conjugation various prodrugs of Propranolol have been synthesized in search of more lipophilic and long acting derivatives. Among them homologous acyl ester prodrugs of Propranolol like *O*-acetyl, *O*-propionyl carboxylic acid ester (Figure-1) were found to have more lipophilicity, more bioavailability and more biological half-life than Propranolol[2]. Hence, prodrug approach may be an effective means of avoiding first pass metabolism of drugs which undergo extensive first pass elimination[3]. In recent days, the chemistry of fused systems has gained significant importance among medicinal chemists especially Indolizines, because of their potential biological applications such as anti-inflammatory, antiviral, analgesic, anti-tumor and anti-HIV activity, anti arrhythmic and anti-hypertensive activities[4] (Figure-2). Therefore, this rekindled our curiosity to synthesize various Indolizinecarboxylic ester derivatives of Propranolol as prodrugs and evaluate them for their chemical stability and anti-hypertensive activity.

Materials and Methods

Experimental: Melting points were measured by using Fischer-Johns melting point apparatus and are uncorrected. IR spectra were recorded on Fourier Transform Infrared Spectrum using Tensor 27 Spectrophotometer as neat liquids or KBr pellets and absorptions are reported in cm^{-1}. ^{1}HNMR spectra were recorded on Bruker AV III 500MHz FT-NMR Spectrometer in appropriate solvents using TMS as internal standard or the solvent signals as secondary standards and the chemical shifts are shown in δ scales. Coupling constants J are expressed in Hertz. Mass spectra were obtained by using in JOEL-D-300 MS Spectrometer (70ev). Reagents and all solvents were analytically pure and were used without further purification. All the experiments were monitored by analytical thin layer chromatography (TLC) performed on silica gel GF254 pre-coated plates. After elution, plate was visualized under UV illumination at 254 nm for UV active materials.

Synthesis of methyl indolizine-1-carboxylate derivatives (5a-c): To pyridine1 (8 mL), ethyl acetate (60 mL) and chloroacetic acid 2 (9.5 g, 100 mmol) was added, stirred for about 2h at 90ºC and cooled in refrigerator. Solution obtained was filtered and precipitate thus obtained was dried, recrystallized from hot methanol to get pyridinium chloride 3. To this obtained pyridinium chloride 3, methyl acrylate / crotonate / cinnamate 4a-c (2.0 g, 50 mmol), triethyl amine (1.5 mL) and manganese dioxide (4.0 g, 80 mmol) in toluene (80 mL) were added, stirred for 15 h at 90ºC in an RBF. The brown / reddish orange oil thus obtained was collected by distillation at 130ºC, washed with water and dried over calcium chloride gave 5a-cin moderate yields (Scheme-1).

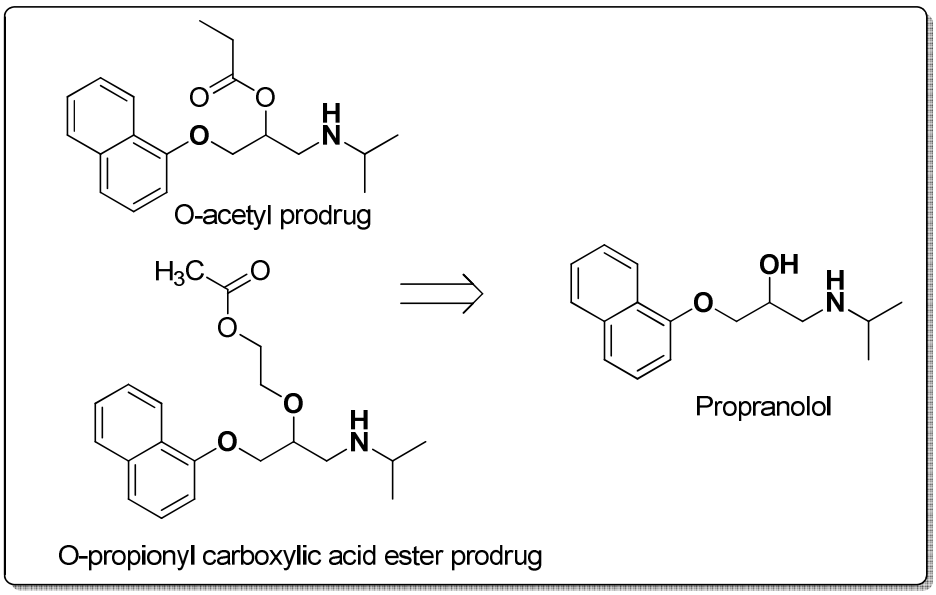


Figure-1
Prodrugs of Propranolol

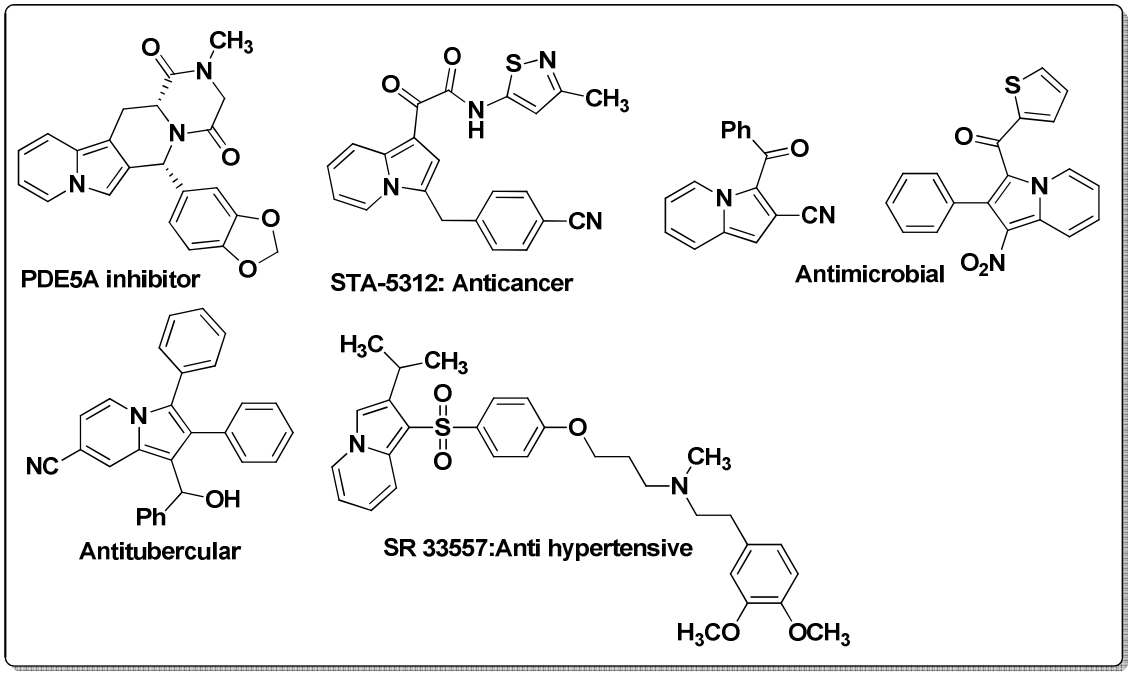
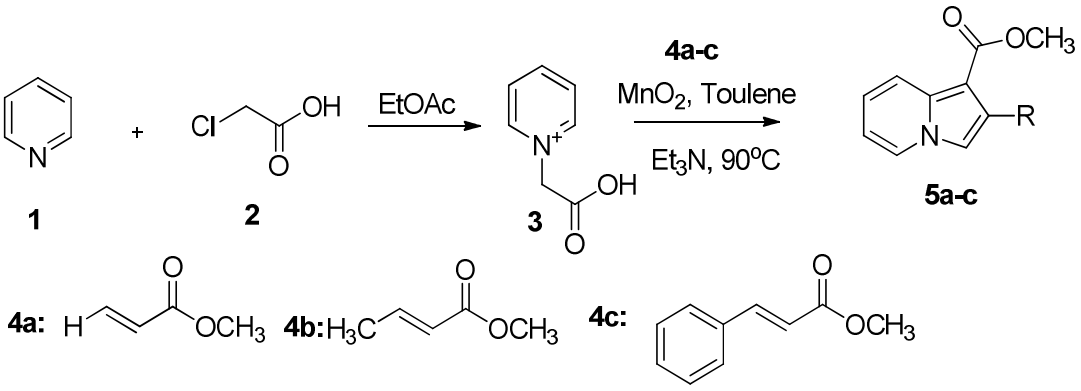


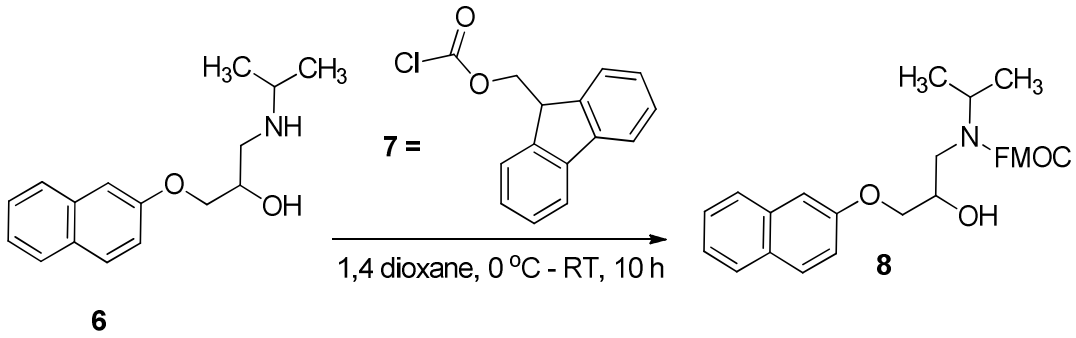
Figure-2
Indolizine based marketed drugs

FMOC protection of propranolol (8): Propranolol 6 (5 g, 19.2 mmol) was dissolved in water and sodium bicarbonate (3.8 g, 38.5 mmol) was added with stirring. The resulting solution was cooled to 5ºC and FMOC chloride 7 (0.01 g, 28.8 mmol) in 1, 4-dioxane was added in cold condition to the above solution. The resulting solution was stirred at 0ºC for 10 h and allowed to warm at room temperature overnight. The reaction mixture was diluted with water and washed with saturated bicarbonatesolution (20mL) and was neutralized with 10% HCl to pH 1 and then partitioned between EtOAc and brine. The organic layer was separated, washed with water, dried over anhydrous Na_2SO_4, concentrated under reduced pressure. The solid8 thus obtained was collected and recrystallized from hot ethanol (Scheme-2).

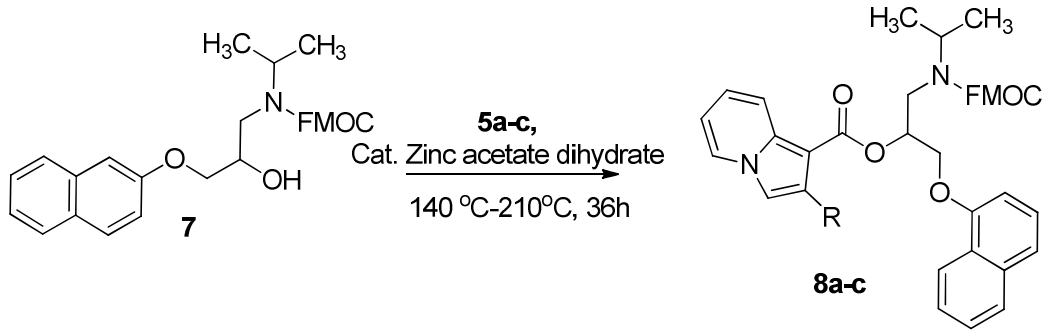
Transesterification derivatives (8a-c): In a two necked RBF equipped with a Dean-Stark apparatus, indolizine derivatives 5a-c (1mL, 4 mmol) and protected Propranolol (1.04 g, 4 mmol) 8 were added. To this, a catalytic amount of zinc acetate dihydrate was added and the reaction mixture was heated to 140-210ºC for 36 hunder inert atmosphere. When methanol began to collect, the molten product thus formed, when cooled immediatelygave8a-c in moderate yields (40 -50%) (Scheme-3).



Scheme-1
Synthesis of methyl indolizine-1-carboxylate derivatives 5a-c



Scheme-2
Protection of amino group in Propranolol



Scheme-3
Synthesis of FMOC protected 3-(naphthalen-1-yloxy) propan-2-ylindolizine-1-carboxylatederivatives 8a-c

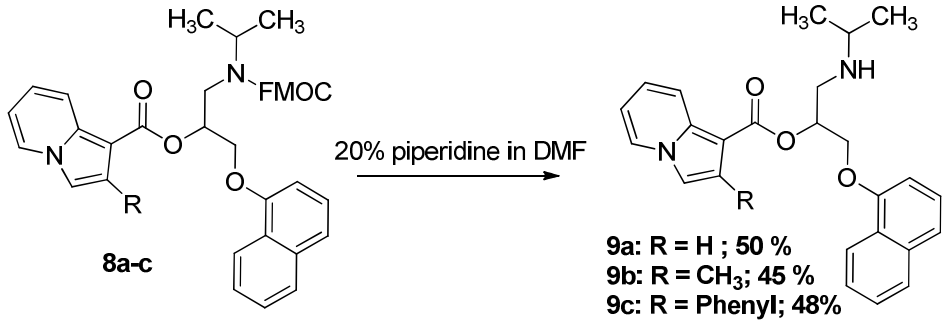
Deprotection of FMOC (9a-c): Above obtained transesterified product8a-cwas dissolved in 10 mL of 20% piperidine in DMF and kept for stirring at room temperature for 24 h and concentrated under reduced pressure. The obtained product9a-cin moderate yields was collected and dried over calcium chloride (Scheme-4).

Characterization: 1- (isopropylamino) -3- (naphthalen-1-yloxy) propan-2-ylindolizine-1-carboxylate (9a):^{1}H NMR (DMSO-*d6*, δ, ppm): δ 8.20-8.36 (m, 3H; Ar-H), 7.50-7.82 (m, 8H; Ar-H), 4.4-5.0 (m, 4H; CH_2), 3.0-3.25 (dd, 4H; C-H), 1.1-1.2 (d, 6H; $2CH_3$); IR (KBr, cm^{-1}): 1102 (C-N), 1731 (C=Ostr), 1573 (C=Cstr), 3393 (N-Hstr), 1017 (C-O-Cstr), 1270 (C-C str), 950 (C-Hstr), 3010 (C-Hstr); MS (ESI)m/z: 403 ($M+H^+$).

***In vitro* chemical stability studies:** The chemical stability studies for synthesized derivatives 9a-cwas performed by using USP-II paddle apparatus at a rotational speed of 50 rpm. 900 ml of pH 6.8 and 7.4 is used as a dissolution media by maintaining temperature at 37±5ºC. 5 mL of the hydrolysis medium was taken out at zero hours and every half an hour up to 3 h for pH 1.2 and up to complete hydrolysis for pH 7.4. 5 mL of the pH buffer was added to the dissolution vessel. The sample withdrawn was analyzed by using UV-spectrophotometer at different maximum wavelength. The amount of drug release was determined by plotting absorbance vs. time graph. By plotting the concentration vs. time in the semi log (semi logarithm) graph paper and slope of the line is calculated which indicate the calculated K (rate constant) value. From the rate constant value "K", hydrolysis half-life of the drug is calculated[5].

Pharmacological Evaluation: Animals of all the groups were fed with 10% fructose solution for 24 days and blood pressure was monitored every day, and on the 24th day the blood pressure was moderately high (140-150 mm per Hg range). On the 26th day, the animals were grouped and drugs were administered according to the dose to respective groups. Blood pressure was recorded as 0-5 h with 30 min interval by tail cuff method[6].The hypertensive animals were divided into four groups (6 each)

Group I: Control – Receives 10% fructose solution. Group II: Standard – Receives Standard Drug Propranolol accordingly. Group III: Receives 9c (15 mg/kg body weight of animal used). Group IV: Receives 9a (15 mg/kg body weight of animal used). After administration of dosage the Systolic Blood Pressure (SBP) was measured by tail-cuff method in each 0 h, 1.0 h, 1.5 h, 2.0 h, 2.5 h, 3.0 h, 3.5 h, 4 h, 4.5 h, 5 h respectively (Table-1). The data obtained were statistically validated by One-way Analysis of Variance (ANOVA)[7] and represented in the following Table-1.



Scheme-4
FMOC Deprotection

Table-1
Systolic Blood Pressure data of Propranolol, Indolizine derivatives 9a and 9c

Compound	0 hrs	0.5hrs	1.0hrs	1.5hrs	2.0hrs	2.5hrs	3.0hrs	3.5hrs	4.0hrs	4.5hrs	5.0hrs
	BP ±SEM	BP ±SEM	BP ±SEM	BP ±SEM	BP ±SEM	BP ±SEM	BP ±SEM	BP ±SEM	BP ±SEM	BP ±SEM	BP ±SEM
Control	141 1.414	141 0.707	141.2 1.281	141.6 1.364	139.4 1.077	141 1.414	141 1.225	142 1.378	141.2 0.860	141.4 0.748	143.2 1.594
Propranolol	140.8 1.068 ns	136.8 0.860 ns	135.2 1.393 *	133.6 0.678 **	128.6 0.927 ***	125.8 1.158 ***	125.6 0.678 ***	127.2 1.562 ***	131.8 1.281 ***	134.8 1.158 *	138.8 1.319 ns
9c	140.2 1.114 ns	140.0 1.828 **	138.2 0.316 ***	133.0 0.400 ***	125.2 1.241 ***	124.7 0.678 ***	121.8 0.066 ***	119.6 0.969 ***	117.5 1.122 ***	116.9 0.509 ***	116.3 0.734 ***
9a	140.8 1.463 ns	139 1.304 ns	136.8 1.158 ns	134.4 1.288 **	130.8 1.934 ***	127.2 2.764 ***	128 2.828 ***	128.6 2.857 ***	131.9 2.449 ***	136.9 2.518 *	139.5 0.927 ns

Control = 10% fructose P = Propranolol (15 mg), One-way Analysis of Variance (ANOVA) ***P<0.001 **P<0.01 *P<0.05 ns P<0.05.

Results and Discussion

The drug release study of Propranolol from the synthesized prodrugs 9a and 9c proved that they hydrolyze significantly at pH 6.8 and 7.4. This proves the stability of prodrug at gastric pH and easily gets hydrolyzed at both blood and intestinal pH, and would be available for the action by significantly eliminating the problem of fast metabolism and local irritation when compared to the parent drug. The % CDR of 9c at pH 7.4 was found to be maximum i.e. 92 % within 3 h and at pH 6.8 it is 86% but at pH 1.2 it is only 8.13 %, whereas in case of 9a the % drug release is 83% at pH 7.4 and 71 % in pH 6.8 but at pH 1.2 it is only 10.6 %. The maximum drug release from the synthesized prodrugs was found at slightly alkaline pH, due to the ester bond fission at alkaline pH and the ester bond was not broken at gastric pH which attributes to low drug release at gastric pH. (Figure-3 and Figure-4)

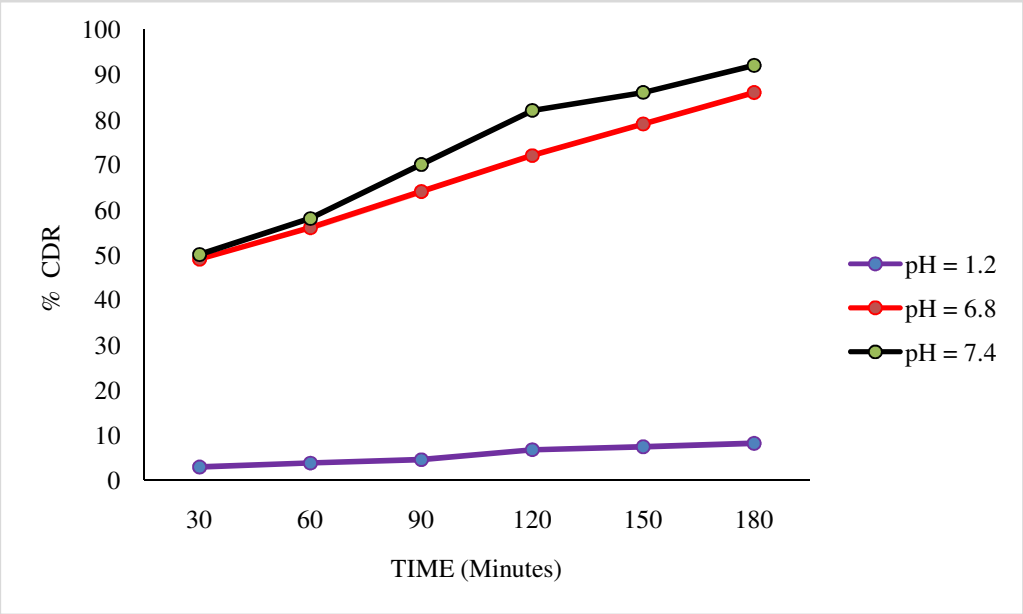


Figure-3
Comparison of %CDR of Propranolol from 9a at pH 1.2,6.8 and 7.4

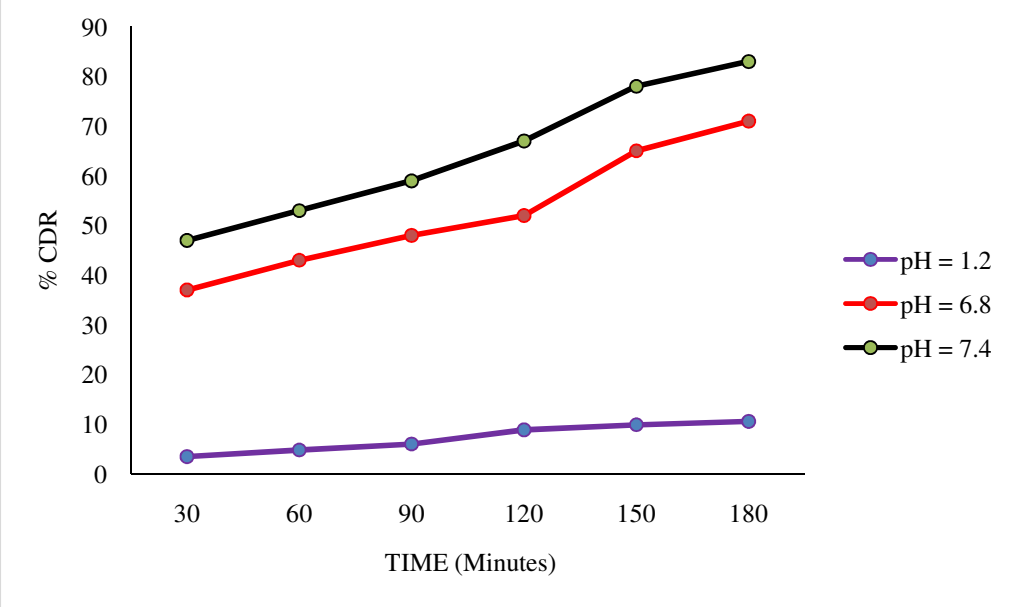


Figure-4
Comparison of %CDR of Propranolol from 9c at pH 1.2,6.8 and 7.4

After analyzing the data of the synthesized compounds9a and 9c, effects were compared with control and standard drug, Propranolol. By considering the recorded Blood pressure data, among the synthesized derivatives, 9c has shown significant anti-hypertensive activity and prolonged duration of action when compared to the standard drug Propranolol but onset of action is slightly delayed than standard drug, which may be due to time required for ester hydrolysis. The reduction in Systolic Blood Pressure in case of 9c is more than that of the standard drug, Propranolol which suggests the probable synergistic effect of 2-phenyl indolizine moiety on hypertension, whereas in case of9a which contains unsubstituted indolizine moiety condensed with Propranolol, there is no much significant reduction in Systolic Blood Pressure, which suggests no synergistic contribution of unsubstituted Indolizine ring on hypertension and its effect is same as standard drug.

Conclusion

The present study was carried out to synthesize different indolizine-1-carboxylate derivatives 9a-cby condensing antihypertensive drug, Propranolol and evaluated for drug release, chemical stability and anti-hypertensive activity. Among all the derivatives 9a has shown significant anti-hypertensive activity and prolonged duration of action when compared to the standard drug Propranolol but onset of action is slightly delayed than standard drug, which may be due to time required for ester hydrolysis.

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