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Synthesis, Hydrolysis Kinetics and Comparative Pharmacological Evaluation of Co-Drugs of Propranolol and Metformin

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ABSTRACT

A codrug is chemically linked together, which cleaves under physiological conditions to regenerate each constituent compound. In present investigation, we have made new co-drugs of Propranolol and Metformin by using Propranolol as the starting compound. Different derivatives of co-drugs were made by reacting Propranolol with Metformin through ester linkage with different anhydrides and the structure and the purity of the derivatives were confirmed by Rf value, MP, TLC, IR, NMR and Mass spectral data. The synthesized compounds were then meant for hydrolysis kinetics studies and screened for antihypertensive and antidiabetic activities. From the hydrolysis and the release study, it was observed that the co-drugs underwent significant hydrolysis in blood (pH 7.4) tissue and has shown delayed onset of action, with respect to the standard drugs. The delayed onset may be due to the hydrolysis of amide linkage followed by the release of the prodrug which finally releases the active drug. The co-drugs have shown significant antihypertensive and antidiabetic activity with respect to the respective standard drugs.

INTRODUCTION

Diabetes often increases the risk of high blood pressure. Hypertension is considered as a common co-morbid condition with diabetes**.** Co-morbidity is the presence of one or more disorders (or diseases) in addition to primary disease or disorders.[1]

Diabetes Mellitus is a group of chronic metabolic diseases characterized by hyperglycemia, in which a high blood glucose concentration is seen.[2]

Hypertension is defined as cardiac chronic condition characterized by elevated systemic arterial blood pressure than normal (120/80mmHg).[3]

In diabetes Polypharmacy is unavoidable. Diabetes can give rise to a wide range of complications such as hypertension, neuropathy, dyslipidemia and depression can also exist, leading to the administration of many medicines for adequate control. **Polypharmacy** indicates many drugs in which problems that occur when a patient is taking more medications than are actually needed.[4] Instead of using multiple drugs, **co-drug** or **mutual prodrug** can be administered as single chemical entity. Co-drug or Mutual prodrug is the type of Carrier-linked prodrug, in which a carrier group is attached to the active drug in order to alter its physicochemical properties followed by subsequent enzymatic or non-enzymatic release of the active drug moiety. The term 'mutual prodrug' or 'co-drug' refers to two or more therapeutic compounds bonded via a covalent chemical linkage. It differs from prodrug in replacement of inactive group by active group, which are coupled directly or indirectly (by a cleavable spacer). When two drugs are administered simultaneously they may not be absorbed or transported well to the target site of action but by the co-drug, this can be achieved, by increase in rate of absorption. As co-drug, two different drugs are linked together with or without spacer and designed to release parent drugs at the desired site of action.[5]

MATERIALS AND METHODS

Materials

Melting points of the compounds were taken by using open capillary tube method and are found uncorrected. IR spectra of the compounds were recorded on Fourier Transform Infrared Spectrum using Tensor 27 Spectrophotometer, Bruker optik (Germany) using ATR technique. The ^{1}H NMR (400 MHz) spectra were recorded on a Bruker AV III 500MHz

FT-NMR Spectrometer (with TMS as internal reference). Mass Spectra were recorded in JOEL D-300 MS Spectrometer (70ev), SHIMADZU (Japan) by LCMS-2010A. The purity of the compounds was checked by Thin Layer Chromatography method on silica gel-G coated plates using ethyl acetate: methanol: ammonia (8:2:1) and visualized under UV chamber. IR, ^{1}H-NMR and Mass spectral data were found consistent with the assigned structures.[6]

Synthetic methods

Step1 Synthesis of Prodrugs of Propranolol P (a-c)

Propranolol, (1.69 mmoles) was stirred at 85-90°C with the anhydride (Succinic/Maleic/ Malonic) (5.00 mmoles) in 1 ml of DMF (Dimethyl formamide) for a period of 6-12 hours. The mixture was then cooled to room temperature and few ml of water was added to the obtained product (O-hemisuccinate/ O-hemimaleate/ O-hemimalonate) and the solution was washed with ether to remove DMF and the amide derivatives (if any). The aqueous solution was then evaporated to yield the products as white precipitates P (a-c).[7]

Step2 Synthesis of Co-drugs of Propranolol and Metformin 2(a-c)

A two-necked RBF was equipped with a Dean-Stark apparatus topped with a reflux condenser and a nitrogen inlet. The vessel was charged with the obtained product P (a-c), boric acid and 20 ml of methanol. To this stirred colorless mixture, Metformin (0.0082 mol.) was added in one portion. The reaction mixture was then refluxed for about 16-18 hours and water was collected in the Dean-Stark trap. The mixture was then poured into DCM (Dichloromethane) which led to the immediate precipitation of a white solid. The obtained product was then dried in vacuum 2a-PSM, 2b-PHM, and 2c-PMM.[8]

SCHEME OF SYNTHESIS

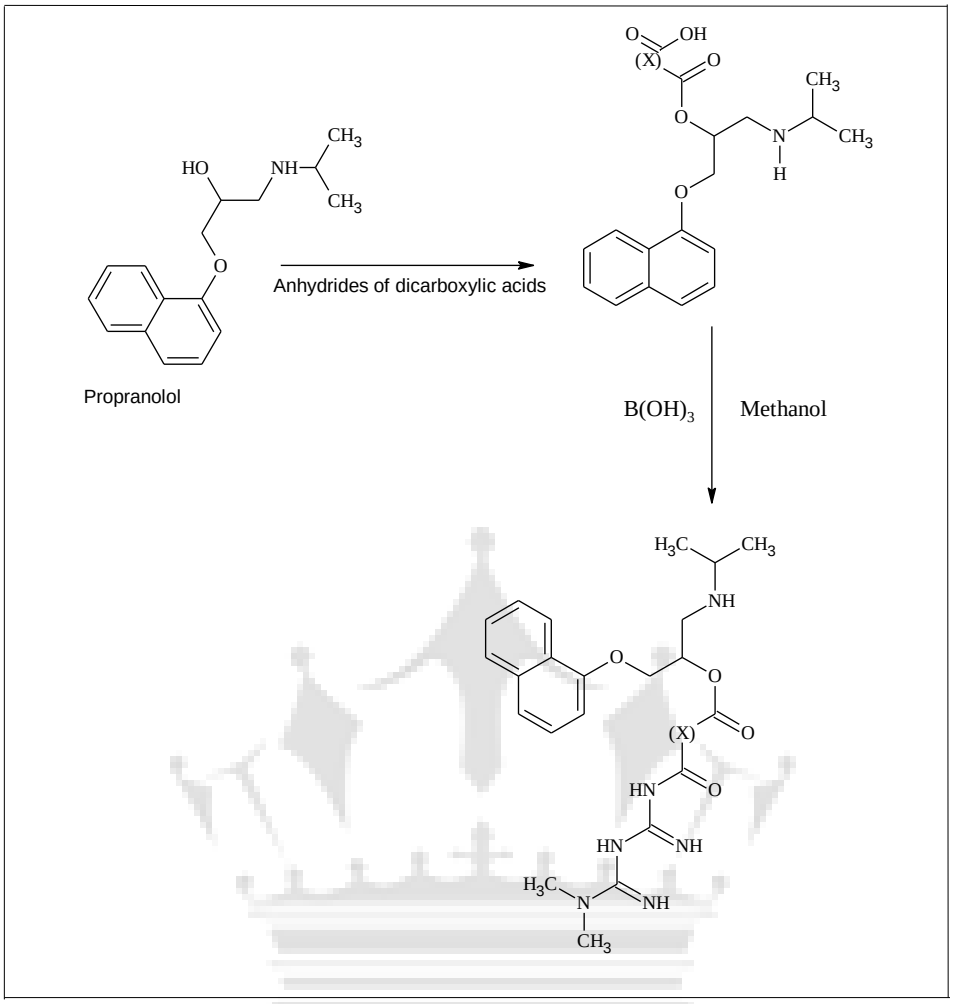

Figure: 1

Step1 Synthesis of Prodrugs of Propranolol

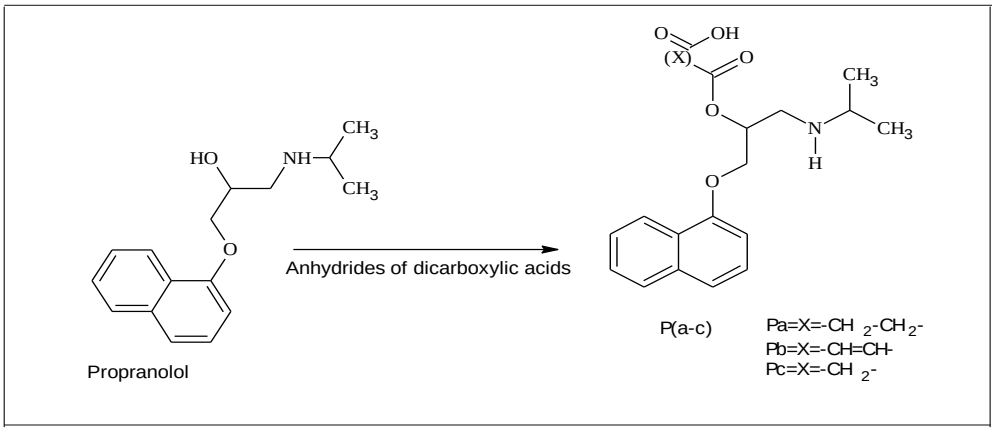

Figure: 2

Step2 Synthesis of Co-drugs of Propranolol

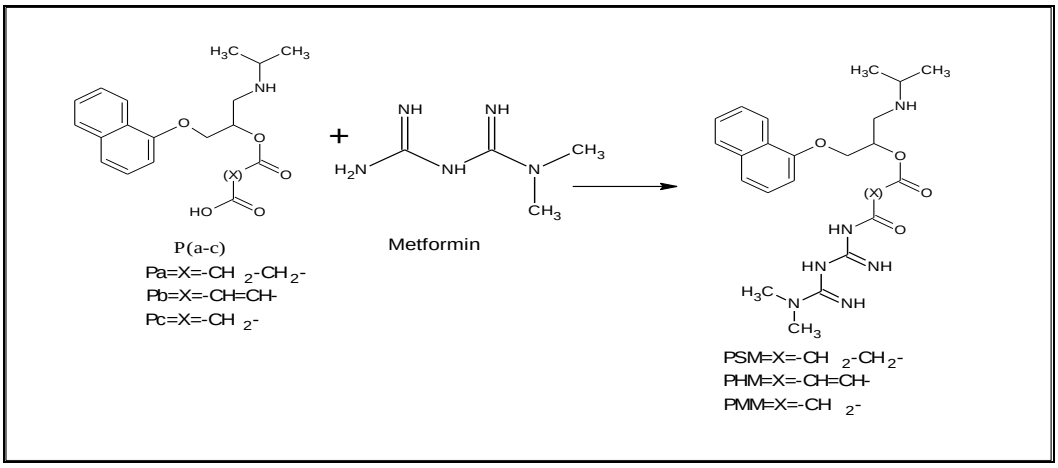

Figure: 3

Hydrolysis Kinetics method

The hydrolysis kinetics of synthesized co-drugs was determined in different pH-buffer solution. The amount of drug release at pH 1.2, 6.8, 7.4 was (physiological solution) determined by UV-Spectrophotometrical method.[9]

Preparation of Standard curve of Propranolol at pH 1.2, 6.8 and 7.4:

2-50 µg/ml of dilution was made in aqueous acidic solution for pH 1.2 and phosphate buffer for pH 6.8, 7.4 and absorbance was taken in reported λ max (290 nm). The absorbance vs. concentration was plotted in graph.

Preparation of Standard curve of Metformin at pH 1.2, 6.8 and 7.4:

2-50 µg/ml of dilution was made in aqueous acidic solution for pH 1.2 and phosphate buffer for pH 6.8, 7.4 and absorbance was taken in reported λ max (233 nm). The absorbance vs. concentration was plotted in graph.

Estimation of drug content: 100 mg of co-drug was dissolved in each 900 ml of (pH 1.2, 6.8, 7.4) buffer solution maintained at 37°C temperature. 5 ml of this solution was pipetted out and made up the volume up to 10 ml. The absorbance was then measured at 290 nm, 233 nm for Propranolol, Metformin respectively.

***In-vitro* Hydrolysis Kinetic studies:** The hydrolysis was performed by using USP-II paddle apparatus at a rotational speed of 50 rpm. 900 ml of pH 1.2, 6.8, 7.4 were used as dissolution

media and temperature was maintained at 37 ±1°C. 5 ml of the hydrolysis medium was taken out at 0 hour and for every half an hour up to 6 hours. After every withdrawal, 5 ml of the buffer solution was added to the dissolution vessel to maintain the volume (900 ml) of dissolution medium. The sample withdrawn was analyzed by UV-spectrophotometer at different λ max. The amount of drug release was determined by plotting % CDR vs. time graph.[10]

PHARMACOLOGICAL EVALUATION

The synthesized compounds were screened for their antidiabetic and antihypertensive activities. Acute oral toxicity studies are carried out to determine the safety dose of the synthesized co-drugs.[11]

ANTI-DIABETIC ACTIVITY by Streptozotocin (STZ) induced method

Male Wistar albino rats (220-250 g) fasted for overnight before challenging with single injection of freshly prepared STZ and injected within 5 min of the preparation in order to prevent degradation at a dose of 35 mg/kg, i.p. After administration of STZ, the animals had access to feed and water *ad libitum*. Development of hyperglycemia in rats was confirmed by fasting blood glucose estimation by 72 hours post STZ injection wherein the animals were made again fasted for about 14 hours before blood collection from tail vein. Rats with fasting blood glucose levels of above 200 mg/dl at 72 hours STZ injection were considered as diabetic and included in the study.[12]

ANTI-HYPERTENSIVE ACTIVITY by Fructose induced method

Animals of all the groups were fed with 10% fructose solution for 24 days and Blood Pressure (BP) was monitored every day and on 24th day, the BP was moderately high (140-150 mm per Hg range). On 26th day, the animals were grouped and drugs were administered accordingly. BP was recorded as 0-5 hours with 30 min interval by tail cuff method**.** The hypertensive animals were divided into five groups (6 each). After administration of dosage, the systolic blood pressure was measured by tail-cuff method in each 0 hour, 1.0 hour, 1.5 hours, 2.0 hours, 2.5 hours, 3.0 hours, 3.5 hours, 4 hours, 4.5 hours and 5 hours respectively.[13]

Evaluation

Since maximum effects on the chosen parameters were achieved after 6 weeks, the duration of treatment could be limited to this time. Statistical analysis was performed using one-way analysis of variance, followed by the Newman-Keuls test.

RESULTS AND DISCUSSION

Table 1: Physicochemical Data of the Synthesized Co-Drugs

Molecular Formula	Compound Code	Mol. Wt. (gm)	M.P. (°C)*	Theoretical Yield (gm)	Practical Yield (gm)	% Yield (%)	R_f Value**
$C_{23}H_{33}N_6O_4$	PSM	457	272	1.58	1.08	68.35	0.70
$C_{23}H_{31}N_6O_4$	PHM	455	288	1.42	0.67	47.18	0.68
$C_{22}H_{32}N_6O_4$	PMM	442	256	1.06	0.6	64.17	0.70

*= Uncorrected

**= Mobile Phase-Methanol: Ammonia (4:0.1)

Chemistry

Spectral details of 2-[(4-{[N, N-dimethylcarbamimidoyl) ethanimidoyl] carbamoyl} butanoyl) oxy]-1-napthalen-2-yloxy)-3-[(propan-2-yl) amino] propan-2-yl (PSM)

IR 1747 (C=O), 3017 (C=C), 3338 (NH stre), 2934 (C-H stre) Cm $^{-1}$; **^{1}H NMR (DMSO)** 8.27 **(**dd,1HAr-H**),** 7.77-7.79 **(**m, 1H,Ar-H**),** 6.29 (s, 1H), 3.2-3.9 (s, 6H), 2.27 (s, CH_3, 3H), 2.87-4.5(m, 4H) ; EI MS (m/z): 429.1($M+H^+$).The mass spectroscopic analysis gives the parent peak confirming molecular weight of the targeted compounds.

Spectral details of 2-[(4-{[N, N-dimethylcarbamimidoyl) ethanimidoyl] carbamoyl} but-2-enoyl) oxy]-1-napthalen-2-yloxy)-3-[(propan-2-yl) amino] propan-2-yl (PHM)

IR 1617 (C=O), 3077 (C=C), 2936 (CH_3), 3339 (NH stre), 2872 (CH_2), 1053(CN stre) Cm $^{-1}$ **^{1}H NMR (DMSO)** 6.9-8.2 (m, Ar-H, 5H)**,** 7.6 (s, 2H, NH_2)**,** 8.3 (s, NH, 1H), 3.3 (s,6H)**,** 4.38 **(**s, 1H**);** EI MS (m/z): 442 .($M+ H^+$).The mass spectroscopic analysis gives the parent peak confirming molecular weight of the targeted compounds.

Table 2: Release of Co-Drugs

Compound code		% Cumulative drug release (Time in hours)													
		0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0
PSM	P	0	11.26	20.15	30.25	45.19	53.17	65.25	73.04	81.25	93.47	97.29	11.26	20.15		
	M	0	0	5.44	10.25	18.62	29.08	41.21	45.02	57.68	63.45	77.25	79.02	85.63	91.56	95.28
PHM	P	0	12.24	21.32	33.42	47.18	60.25	67.12	77.26	85.63	90.38	96.91				
	M	0	0	5.15	11.26	17.62	23.12	30.19	42.32	50.62	67.45	73.82	78.54	84.02	90.25	95.26

Biological Results

Anti-diabetic Activity

Table 3: Effect of Co-drugs on fasting serum glucose levels of rats

Groups	Dose administered (mg/kg)	Serum glucose levels in mg/dl (Mean ± SEM)			
		0 day	6 day	12 day	18 day
Group I	Normal saline 10ml/kg, p.o.	107.66 ± 3.77	107.66 ± 3.19	106.50 ± 3.98	108.50 ± 4.32
Group II	Metformin 500mg/kg, p.o.	344.00 ± 11.78	327.16 ± 8.86	258.33 ± 12.81	136.33 ± 3.26
Group III	PSM 100mg/kg, p.o.	340.50 ± 1.47*	336.43 ± 3.33*	302.55 ± 7.21**	258.54 ± 3.30**
Group IV	PHM 100mg/kg, p.o.	345.50 ± 2.07*	338.12 ± 1.47*	304.33 ± 1.47**	250.12 ± 2.65**

All the groups are compared with disease control.

*p≤0.01 Diabetes vs. Normal control

**p≤ 0.01 Metformin/PSM/PHM vs. Diabetic control

Antihypertensive Activity

Table 4: Systolic Blood pressure data of Synthesized Co-drugs

Sl No	Compound	0 hr	0.5hr	1.0hr	1.5hr	2.0hr	2.5hr	3.0hr	3.5hr	4.0hr	4.5hr	5.0hr
		BP ±SEM	BP ±SEM	BP ±SEM	BP ±SEM	BP ±SEM	BP ±SEM	BP ±SEM	BP ±SEM	BP ±SEM	BP ±SEM	BP ±SEM
1	**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
2	**P**	142.8 ±1.068	136.8 ±0.868	135.2 ±1.393	133.6 ±0.678	128.6 ±0.927	125.8 ±1.158	121.4 ±0.678	127.2 ±1.562	131.8 ±1.281	134.8 ±1.158	138.8 ±1.139
3	**PSM**	146.8 ±0.860 ns	144.1 ±1.049 **	138.2 ±0.583 ***	136.4 ±1.049 ***	128.2 ±0.583 ***	127.6 ±1.077 ***	125.3 ±0.547 ***	123.4 ±0.509 ***	125.3 ±0.632 ***	129.8 ±1.020 ***	132.8 ±0.860 ***
4	**PHM**	143.4 ±1.077 ns	140.2 ±0.374 ns	138.4 ±0.509 ***	132.2 ±0.663 ***	126.2 ±0.800 ***	126.8 ±0.503 ***	121.3 ±0.894 ***	121.8 ±0.860 ***	127.8 ±1.049 ***	131.8 ±0.583 ***	135.4 ±1.030 ***

Control = 10% fructose, **P** = Propranolol (15 mg), **M** =Metformin (20 mg), **PSM** (56.5 mg), **PHM** (56.5 mg), hr = hour

One-way Analysis of Variance (ANOVA)

***P<0.001 **P<0.01 *P<0.05 ns P<0.05 Ns P>0.05

CONCLUSION

The objective of the present work was to synthesize few co-drugs of Metformin and Propranolol and to carry out the *In-vitro* hydrolysis kinetic study and comparative antidiabetic and antihypertensive activities.

Different derivatives of co-drugs were made by reacting Propranolol with Metformin through ester linkage with three different anhydrides and the structure and the purity of the derivatives were confirmed by Rf value, MP, TLC, IR, NMR and Mass spectral data. Hydrolysis kinetics was carried out using USP-II paddle dissolution apparatus at different pH 1.2, 6.8, 7.4 to find out release profile of active drugs and it was observed that both co-drugs were found to be stable at gastric and intestinal pH but unstable and hydrolysable at systemic pH 7.4 i.e. in blood. All the synthesized co-drugs were screened for *In-vivo* antidiabetic and antihypertensive activities. LD_{50} of the co-drugs was determined as per the OECD guidelines. Of the three, two co-drugs (PSM, PHM) have shown delayed onset and more prolonged anti-diabetic action than the standard drug Metformin but the antihypertensive activity was as same as that of the standard drug Propranolol with respect to duration of action. *In-vivo* enzymatic hydrolysis and the comparative pharmacological evaluation of a physical mixture containing Propranolol and Metformin will be taken up in the future.

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