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Synthesis, Hydrolysis Kinetics and Comparative Pharmacological Evaluation of Co-Drugs of Metformin and Anti-Hypertensive Agents

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ABSTRACT

Metformin has been combined chemically with antihypertensive agents like Propranolol and Furosemide by Williamson's ether synthesis and direct amidation respectively with the objective of obtaining single chemical entity in the form of co-drugs. Structures and physicochemical parameters of all synthesized co-drugs were established by MP, BP, TLC, IR, ^{1}H NMR and Mass spectral data. The co-drugs were then subjected to hydrolysis kinetics at different pH (1.2, 6.8 and 7.4). The drug release study of Metformin, Propranolol and Furosemide from the synthesized co-drugs proved that they hydrolyze significantly at pH 7.4 and are remain unhydrolysed at pH 1.2 and 6.8. This proves the stability of both the co-drugs at gastric and intestinal pH and easily gets hydrolyzed in blood. The co-drugs were then screened for their antidiabetic activity and antihypertensive activity and compared with parent drugs. Both the co-drugs PMCD, FMCD have shown significant antidiabetic activity with respect to duration as well as intensity of action. Co-drug of Metformin and Propranolol (PMCD) has shown delayed release and more prolonged antihypertensive action when compared to standard drug Propranolol where as co-drug of Metformin and Furosemide (FMCD) has shown immediate release and duration of antihypertensive action same as that of standard drug Furosemide.

Keywords: Metformin, Propranolol, Furosemide, co-drugs, antidiabetic, antihypertensive.

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INTRODUCTION

Diabetes and high blood pressure are closely related disorders.[1] They occur together so frequently that they are officially considered to be **"co-morbidities".**

Hypertension is defined as cardiac chronic medical condition in which systemic arterial blood pressure is elevated than normal (120/80mmHg).[2]

Diabetes Mellitus and hypertension are common disorders that co-exist at a greater frequency than chance alone would predict. Hypertension in diabetic individuals markedly increases the risk and accelerates the course of cardiac disease, peripheral vascular disease, stroke, retinopathy and nephropathy. Diabetic individuals with co-existing hypertension have a much greater prevalence of stroke and transient ischemic episodes.[3]

To overcome these complications involved in co-existence of diabetes and hypertension, apart from lifestyle modification it requires the use of multiple medications in combination which is nothing but **Polypharmacy**.[4]

Polypharmacy means "many drugs" and refers to problems that can occur when a patient is taking more medications than are actually needed.[5]

So instead of using multiple drugs separately in same or different forms, these drugs may be administered as single chemical entity as **co-drug** or **mutual pro-drug.** Co-drug or Mutual prodrug is the type of Carrier-linked prodrug, where the carrier linked prodrug consists of the attachment of a carrier group to the active drug to alter its physicochemical properties and then subsequent enzymatic or non enzymatic mechanism to release the active drug moiety. The term 'co-drug' or 'mutual prodrug' refers to two or more therapeutic compounds bonded via a covalent chemical linkage. Regardless of being similar to prodrug it differs in having inactive group replacement by active group, which are coupled directly or indirectly (by a cleavable spacer).[6] When two drugs are administered simultaneously they may not be absorbed or transported well to the target site of action but the co-drug has improved absorption rate and can be easily transported to the target site of action. It has to be stable at the gastrointestinal level, but then it has to be hydrolyzed to provide two (or more) active drugs.[7]

MATERIALS AND METHOD

Materials

The melting points were taken in open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on Fourier Transform Infrared Spectrum using Tensor 27Spectrophotometer, 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 using silica gel-G coated plates using ethyl acetate: methanol: ammonia (8:2:1) and visualized in UV chamber. IR, ^{1}H-NMR, ^{13}C NMR, Mass spectroscopy and were consistent with the assigned structures.

SYNTHETIC METHODS

Scheme 1 Co-drug of Propranolol and Metformin

Step 1 Synthesis of 2-chloro-N-[N-(N, N-dimethyl carbamimidoyl) carbamimidoyl) acetamide (Prodrug of Metformin).

To an n-butanolic solution (15ml) of metformin hydrochloride (1 eq), chloroacetyl chloride (1 eq) was added drop wise and refluxed for 14 hours at 70˚C. Evaporated the excess of solvent, the product obtained was dried and recrystallized from ethanol.

Step 2 Synthesis of 2-({[(1, 1-dimethyl carbamimidamido) methamimidoyl] carbamoyl} methoxy)-1-(naphthalen-1-yloxy)-3-[(propan-2-yl) amino] propan-2-yl.

To the solution of Propranolol HCl (1 eq) in DMF (25ml) in a round bottom flask, finely powdered K_2CO_3 (2.1 eq) was added and mixed properly. Then metformin prodrug (1.6 eq) was added and refluxed with stirring for 40 hours.

Scheme 2 Co-drug of Metformin and Furosemide

Synthesis of 4-chloro-N-[N-(N, N-dimethylcarbamimidoyl) carbamimidoyl]-2-[(furan-2-yl methyl)amino]-5-sulfamoylbenzamide.

Furosemide (1 eq), boric acid (0.1 eq) and 20 ml of 1, 4-dioxane are added to a 2-necked RBF equipped with dean-stark trap and a nitrogen inlet. To the colorless reaction mixture was added metformin (1.3 eq) in one portion and was allowed to reflux for 36 hours. After the mixture was allowed to cool to ambient temperature, it was poured into 50 ml of n-hexane with continuous stirring till oil got collected at the bottom. The obtained oily layer was collected by decanting n-hexane and distilled water was added to oily layer leading to precipitation of brown mass which was filtered. The filtered product was dried over $CaCl_2$ and recrystallized from hot ethanol.

SCHEME

Scheme 1 Co-drug of Propranolol and Metformin

Step 1

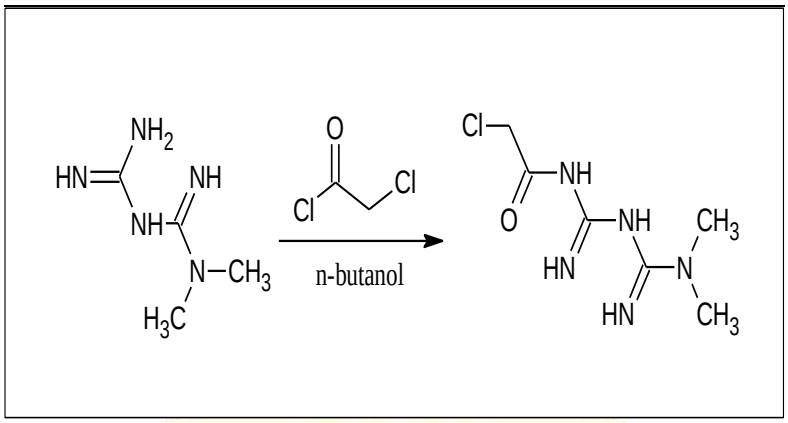

Figure: 1

Step 2

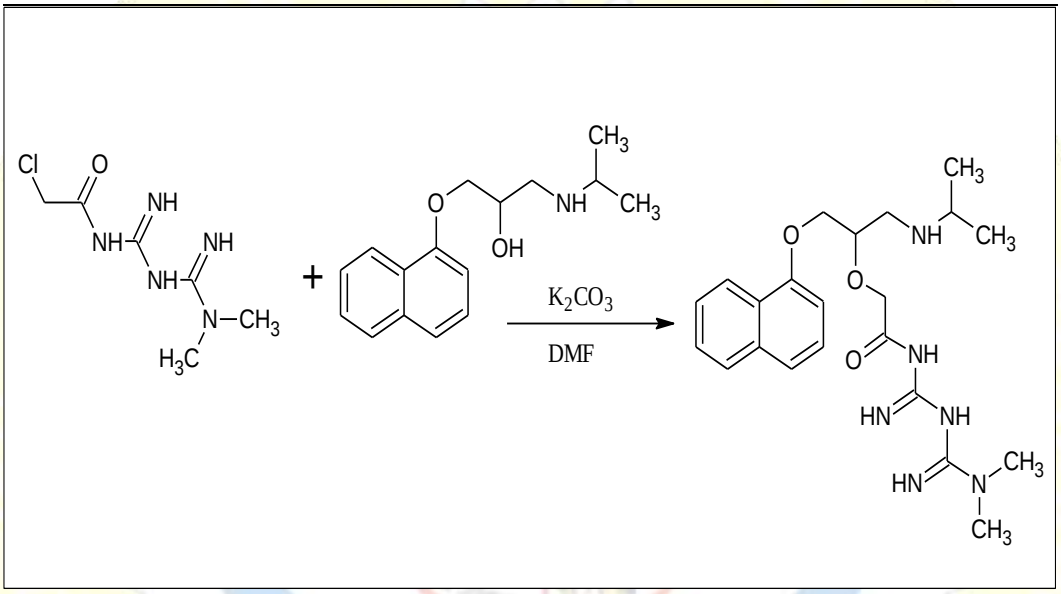

Figure: 2

Scheme 2 Co-drug of Metformin and Furosemide

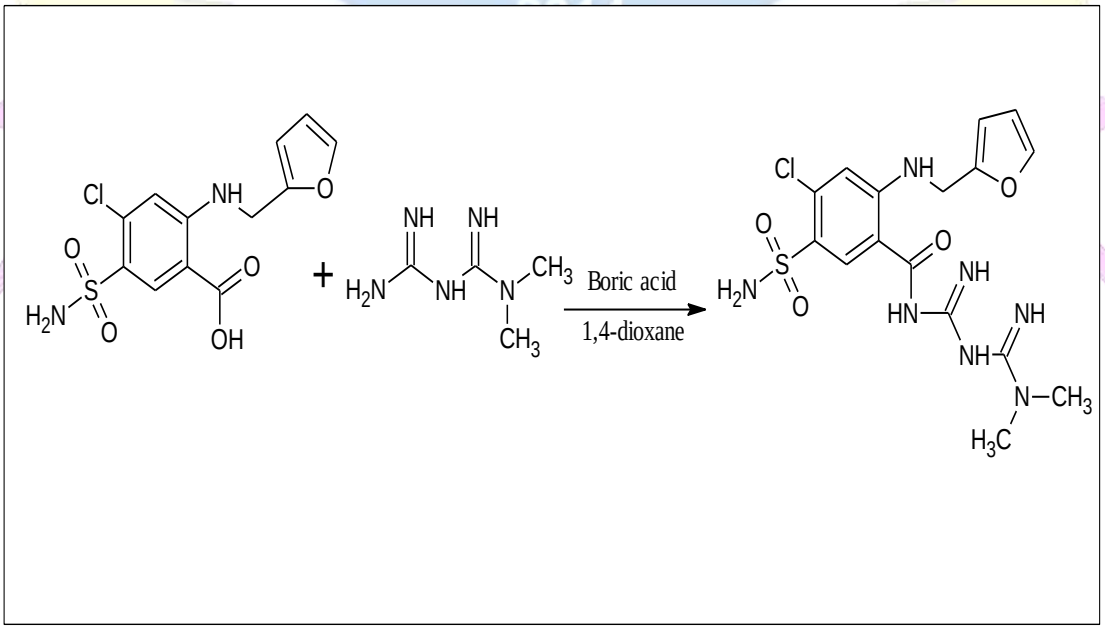

Figure: 3

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.

Estimation of drug content:

100 mg of co-drug was dissolved in each (pH 1.2, 6.8, 7.4) 900 ml buffer solution maintained at 37°C temperature. 5 ml of this solution was pipette out and made up the volume up to 10 ml. The absorbance was then measured at 290 nm, 233 nm and 274 nm for Propranolol, Metformin and Furosemide 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.

PHARMACOLOGICAL EVALUATION

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

The synthesized co-drugs as various dose level like (50, 300, 500, 1000) mg/kg body weight suspended in 5% DMSO were administered as a single dose to a pair of mice. As per dose level orally administered to a group of 10 mice of both sexes about equal in number which have the treated mice were observed continuously for 2 hours and then occasionally for 4hours and finally overnight mortality was recorded. LD50 was determined as 500 mg/kg for PMCD and 1000 mg/kg for FMCD.[8]

Experimental method

Male Wistar albino rats (220-250 g) were fasted for overnight before challenging with single injection of STZ, freshly prepared and injected within 5 min of preparation to prevent degradation at a dose of 35 mg/kg, i.p.[9]After administration of STZ the animals had access to feed and water *ad libitum* .The development of hyperglycemia in rats was confirmed by fasting blood glucose estimation 72 hours post STZ injection wherein the animals were fasted again for 14 hours before blood collection from tail vein. The 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.

Screening

By Fructose induced method

Animals of all the groups were fed with 10% fructose solution for 24 days and BP was monitored every day, and on the 24th day the BP 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. BP was recorded as 0-5 hours with 30 min interval by tail cuff method.[10]

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 hour, 2.0 hours, 2.5 hours, 3.0 hours, 3.5 hours, 4 hours, 4.5 hours and 5 hours respectively.

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-Keul's test.[11]

RESULTS AND DISCUSSION

Table 1: Physicochemical Data of the Synthesized Co-Drugs

Compound code	Molecular Formula	Mol.Wt.(g)	M.P. (ºC)*	% Yield(%)	R_f Value**
PMCD	$C_{22}H_{32}N_6O_3$	428.5	195	70.05	0.7
FMCD	$C_{16}H_{20}ClN_7O_4S$	441.8	152	62.68	0.65

Chemistry

FT-IR spectrum details of 2-({[(1,1-dimethyl carbamimidamido) methamimidoyl] carbamoyl} methoxy)-1-(naphthalen-1-yloxy)-3-[(propan-2-yl) amino] propan-2-yl(PMCD)

IR 1652(C=O), 3145 (C=C), 2873 (CH_2), 3332 (NH stre), 1066 (CN stre), 1575 (NH bend), 2930 (C-H stre), 1192(COC) Cm $^{-1}$ **^{1}H NMR (DMSO)** 8.27 **(**dd,1HAr-H**),** 7.77-7.79 **(**m, 1H,Ar-H**),** 7.40 (m, 3H, Ar-H), 6.91 **(**dd, 1H, Ar-H**),** 5.4-6.10(s, 2H), 3.2-3.9 (s, 4H), 2.87(s, 6H), 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.

FT-IR spectrum details of 4-chloro-N-[N-(N,N-dimethylcarbamimidoyl) carbamimidoyl]-2-[(furan-2-yl methyl)amino]-5-sulfamoylbenzamide(FMCD)

IR 1696 (C=O), 3109 (C=C), 2930 (CH_3), 3471 (NH stre), 742 (C-Cl), 3334 (NH_2), 2872 (CH_2), 1329 (S=O), 1053(CN stre) Cm $^{-1}$ **^{1}H NMR (DMSO)** 8.64 **(**s, 1H**),** 7.57 **(**dd, 1H**),** 6.36-6.42 (m,2H), 4.52 **(**d, 4H**),** 4.38 **(**s, 1H**),** 4.28 (s, 1H), 3.09 (s, 1H), 2.87 (s, 5H) ; 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 PMCD and FMCD in pH 7.4

Compound code		% Cumulative drug release (Time in hour)												
		0.0	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**	**4.0**	**4.5**	**5.0**	**5.5**	**6.0**
PMCD	P	0	7.53	11.56	15.21	24.66	32.12	45.31	58.91	71.23	79.22	85.31	87.23	90.21
	M	0	18.23	45.16	58.26	65.54	71.86	80.13	87.32	94.88				
FMCD	F	0	13.27	22.67	39.52	53.12	69.26	82.25	91.23	95.16				
	M	0	17.53	28.16	36.25	51.92	67.86	79.31	87.42	93.18				

P = Propranolol **F** = Furosemide **M** = Metformin

BIOLOGICAL RESULTS

Anti Diabetic Activity

Table 3: Effect of synthesized co-drugs on blood glucose levels in STZ induced diabetic rats

Groups	Dose administered	Blood glucose levels (Mean ± S.E.M) in mg/dl		
		0 Day	**3 Day**	**6 Day**
Disease control	Normal saline, 10 ml/kg, p.o.	512.66±27.21	446±27	355.5±84.13
Standard group	Metformin 250 mg/kg p.o.	456±42.3***	107.5±19.44***	104.83±10.33***
PMCD treated group	PMCD 50 mg/kg, p.o.	461.83±42.99***	174.16±38.80***	146.83±20.44***
FMCD treated group	FMCD 100 mg/kg, p.o.	495.83±65.42ns	184±13.22**	170.33±18.48**

All the groups are compared with disease control

***P<0.001= very significant

**P<0.001= significant

*P<0.001= slightly significant

P>0.001=non-significant (ns)

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**	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
3	**F**	148.4 1.435 *	132 1.049 **	124.4 0.927 ***	121 0.547 ***	114.6 0.509 ***	114.2 1.114 ***	107.8 1.020 ***	106 1.095 ***	123.4 0.927 ***	126.8 0.738 ***	132.8 1.158
4	**PMCD**	141.4 1.077 ns	140.2 0.374 ns	137.4 0.509 ***	132.2 0.663 ***	126.2 0.800 ***	123.8 0.503 ***	117 0.894 ***	120.8 0.860 ***	129.2 1.049 ***	130.8 0.583 ***	135.4 1.030 ***
5	**FMCD**	146.8 0.860 ns	140.22 1.049 **	138.2 0.583 ***	124 1.049 ***	121.2 0.583 ***	114.6 1.077 ***	110 0.547 ***	110.4 0.509 ***	110 0.632 ***	134.8 1.020 ***	136.8 0.860 ***

Control = 10% fructose **P** = Propranolol (15 mg) **F** =Furosemide (20 mg)

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 antihypertensive agents of various categories and to carry out the *in vitro* hydrolysis kinetic study and comparative antidiabetic and antihypertensive activities.

Co-drugs of Propranolol and Metformin (PMCD), Furosemide and Metformin (FMCD) have been synthesized successfully.

All the synthesized co-drugs were characterized by physical data like MP, TLC and analytical data such as FT-IR, ^{1}H NMR, and Mass spectra.

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. Both the co-drugs (PMCD and FMCD) have shown significant antidiabetic activity which is comparable with that of standard drug Metformin with respect to duration as well as intensity of action. Co-drug of Metformin and Propranolol (PMCD) has shown delayed release and more prolonged antihypertensive action when compared to standard drug Propranolol where as co-drug of Metformin and Furosemide (FMCD) has shown immediate release and duration of antihypertensive action same as that of Furosemide.

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