Asian Journal of Pharmaceutical Research and Development
(An International Peer-Reviewed Journal of Pharmaceutical Research and Development)

Open Access Available online at http://ajprd.com/index.php Research Article

CHEMICAL HYDROLYSIS, *INSILICO* PHYSICOCHEMICAL PROPERTIES AND *IN-VIVO* PHARMACOLOGICAL EVALUATION OF ANTIDIABTIC AND ANTIHYPERTENSIVE CODRUGS

Ganesh S. Andhale*, Giles D, Suresh Janadri, Basavraj Metikurki

[1]Department of Pharmaceutical Chemistry, Acharya & B M Reddy College of Pharmacy, Bangalore
[2]Department of Pharmacology, Acharya & B M Reddy College of Pharmacy, Bangalore
[3]Department of Pharmaceutical Chemistry, Vivekananda College of Pharmacy, Bangalore

ABSTRACT

In the present research work codrugs of Propranolol and Metoprolol with Metformin were synthesized with an aim of improving the biological activity and to check the effectiveness of release of the parent drugs in presence of spacer. Ester prodrugs of Propranolol and metoprolol were synthesized by using succinic, maleic, pthalic anhydride and substituted derivatives of them. The resulted prodrugs were converted into codrugs **(CO1-6)** by reacting with Metformin. The compounds were characterized by melting point, FT-IR, ^{1}H-NMR and mass spectroscopy. The chemical hydrolysis of **CO1-6** was carried out at the pH 1.2, 6.8 and 7.4. Almost all compounds showed encouraging chemical stability at pH 1.2 and 6.8 whereas showed the moderate hydrolysis at pH 7.4 but codrug **CO4** didn't showed hydrolysis at any pH condition. Further codrugs were screened for antihypertensive and antidiabetic activity by simultaneous induction of two-kidney, one clip renal hypertension and STZ-induced diabetic model in wistar rats. The spacer containing dipropyl maleate codrugs **(CO3 and CO6)** showed longer duration of action. The current paper also describes about the molecular properties evaluation of the synthesized codrugs.

KEY WORDS: propranolol, metoprolol, chemical hydrolysis, metformin, codrugs, antihypertensive, antidiabetic

Article Info: Received: 24Oct 2018; **Review Completed**: 25Nov 2018; **Accepted:**30Nov 2018**;Available online**:15Dec Oct,2018

Cite this article as:
Andhale Ganesh S. *, Giles D, Suresh Janadri, Basavraj Metikurki, Chemical Hydrolysis, Insilico Physicochemical Properties And IN-VIVO Pharmacological Evaluation of Antidiabtic and Antihypertensive Codrugs, Asian Journal of Pharmaceutical research and Development.**2018;6 (6): 36-43**

DOI: http://dx.doi.org/10.22270/ajprd.v6i6.452

***Address for Correspondence**
Ganesh S. Andhale, Department of Pharmaceutical Chemistry Acharya & B M Reddy College of Pharmacy, **Bangalore, India**

INDRODUCTION

Beta blockers (Propranolol and Metoprolol) are mainly used in the treatment of heart disease and related conditions. These drugs reduce blood pressure and manage cardiac arrhythmias and are cardioprotective after myocardial infarction (heart attack)[1]. Beta-1 receptors are present in the kidneys, where they control the release of the hormone renin, which increases blood pressure, so beta-1 blockade of kidney receptors reduces blood pressure[2]. Over decades of clinical use, beta blockers have demonstrated good safety in patients of all ages.

A major problem associated with Propranolol and Metoprolol is their high first passes metabolism and consequent poor systemic availability following oral administration.

The antidiabetic drug Metformin, biguinidine class drug is the choice of drug for patients with obesity and/or hyperlipidemic NIDDM. Along with antidiabetic effect Metformin also possesses good effects on dyslipidemia, hypertension, vascular function and fibrinolytic activity which are absolutely advantageous to patients with NIDDM, the major risk factor group of atherosclerosis or cardiovascular diseases. However Metformin has some drawbacks associated with it such as incomplete absorption from the upper intestine, rapid kidney excretion and suffers from low bioavilabilty and some side effects associated with gastrointestinal tract[3-6].

Increased level of blood pressure is responsible for diabetic microvascular and macrovascular problems. Providentially, If blood Pressure reduces it also reduces the complications associated with it and vice versa. β-blockers can be useful in many patients with diabetes

because they can reduce cardiovascular morbidity and mortality in persons with atherosclerotic cardiac disease. Diabetes and high blood pressure are strongly associated diseases. They occur together so frequently that they are officially considered to be "comorbidities". Coexistence of diabetes and hypertension in human is linked with cardiovascular risk and mortality [7-8.]

Various diseases are treated by a combination of therapeutic agents that are co-administered in separate dosage forms. However, there are potential advantages in delivering the co-administered agents as a single chemical entity. For example, improved delivery and pharmacokinetic properties compared to a physical mixture of the two drugs, and improved targeting of the drugs to site of action[9.]

The designing of prodrug has given the success to overcome the undesirable properties associated with the existing drug. Codrug concept is one step ahead as it minimizes the side effects along with increase in activity. Codrug consists of two pharmacologically active agents coupled together so that each can act as a promoiety for the other agent and vice versa. It is an important area of research, and its introduction in human therapy has given successful results in improving the clinical and therapeutic effectiveness of drugs suffering from some undesirable effects that otherwise hinder their clinical applications[10].

The purpose of designing the codrugs of propranolol and Metoprolol with Metformin is to conquer the limitations like high first pass metabolism, slow and incomplete absorption from GIT which causes abdominal discomfort and pain, nausea, vomiting, diarrhea, anorexia, and metallic taste. In this regard the codrug molecules were specifically designed to determine if such codrugs could improve pharmacotherapy for both hypertension and diabetes, by providing a single, therapeutically effective, codrug dosage form to treat co-morbid conditions of hypertension and diabetes.

MATERIALS AND METHODS:

Propranolol and Metoprolol was obtained as gift samples from Cadila Healthcare Limited, Ahmadabad (India). All solvents were of analytical grade and distilled before use. All the reactions were carried out with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Melting points were determined by open capillary tubes and were uncorrected. FTIR spectra of the powdered compounds were recorded using ATR on a Bruker FTIR spectrophotometer and are reported in cm^{-1} and ^{1}H NMR spectra were recorded on a Bruker (400 MHz NMR) spectrophotometer using TMS as an internal reference (Chemical shift represented in δ ppm). Mass spectra were recorded on GC–MS QP5050A System (benchtop quadrupole mass spectrophotometer). Purity of the compound was checked on TLC plates using silica gel G as stationary phase and was visualized using iodine vapors or under UV chambers. In silico studies were carried out by using Accelrys drug discovery studio.

Synthetic studies

Propranolol prodrugs and codrugs were prepared according to the method described by Andhale GS et al., 2018[11] and Metoprolol prodrugs and codrugs were prepared according to the method described by Andhale GS et al., 2018[12.]

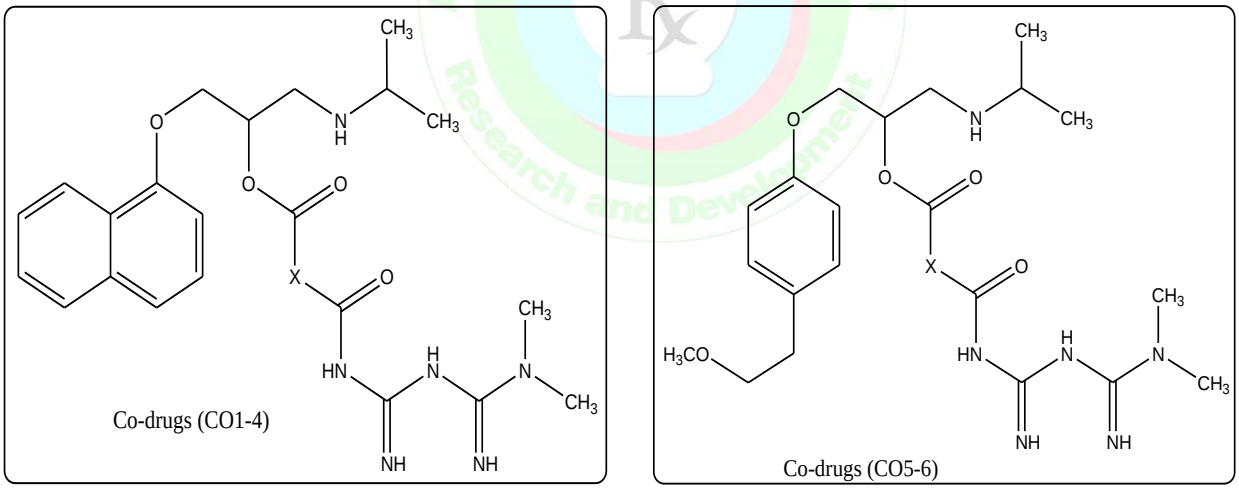
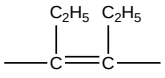
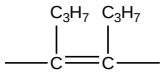
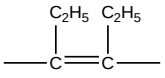
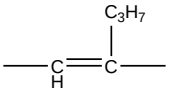
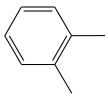
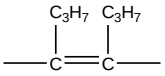


Figure. 1. General structure of codrugs (CO1-6)

Code (CO1-6)	*X*	*Code (CO1-6)*	*X*	*Code (CO1-6)*	*X*
CO1		CO3		CO5	
CO2		CO4		CO6	

Where, X= substituent's used at in the synthesis showed as follows.

Physicochemical and spectral characterization of (CO1-6)

1-(isopropylamino)-3-(naphthalen-1-yloxy)propan-2-yl-3-((N-(N,N-dimethylcarbamimido yl)carbamimidoyl)carbamoyl)-2-ethylpent-2-enoate (CO1): Yield: 88%; mp 257°C. FTIR (KBr) cm^{-1}: 3371.76 (N-H stre), 3025.90 (Ar. C-H stre.), 2938.68 (Ali. C-H stre.), 1699.85 (C=O stre.), 1611.26 (C=N stre.), 1530.40 & 1441.23 (Ar C=C stre.), 1244.44 (C-O-C stre.), 1115.39 (C-N). ^{1}H NMR (DMSO, δ ppm) 8.260 (s, 1H, NH), 7.193-8.244 (m, 7H, Ar-H), 6.680 (s, 1H, =NH), 6.494 (s, 1H, =NH), 4.396 (s, 1H, NH), 4.157-4.170 (d, 2H, CH_2), 3.234- 3.369 (m, 1H, CH), 3.001-3.055 (t, 2H, CH_2), 2.916 (s, 6H, $(CH_3)_2$), 2.481 - 2.499 (m, 1H, CH), 2.069 (s, 1H, NH), 1.815-1.894 (m, 4H, $2CH_2$), 1.514-1.528 (t, 6H, $2CH_3$), 1.265-1.296 (d, 6H, $(CH_3)_2$); ^{13}C NMR (DMSO, δ ppm) 18.201, 18.644, 37.266, 38.884, 39.093, 39.302, 39.510, 39.719, 39.928, 40.137, 46.891, 49.843, 65.293, 69.970, 105.291, 120.213, 121.745, 124.874, 125.241, 126.153, 126.488, 127.412, 128.788, 130.667, 133.064, 134.006, 153.749, 159.164, 168.547; m/z 526.07 (M^+).

1-(isopropylamino)-3-(naphthalen-1-yloxy)propan-2-yl-3-((N-(N,N-dimethylcarbamimidoy l)carbamimidoyl)carbamoyl)hex-2-enoate (CO2):

Yield: 86%; mp 251°C. FTIR (KBr) cm^{-1}: 3389.54 (N-H stre), 3064.91 (Ar. C-H stre.), 2935.58 (Ali. C-H stre.), 1687.33 (C=O stre.), 1575.49 & 1486.47 (Ar. C=C stre.), 1297.97 (C-O-C stre.), 1189.45 (C-N); ^{1}H NMR (DMSO, δ ppm) 8.162 (s, 1H, NH), 7.097-7.850 (m, 7H, Ar-H), 6.813 (s, 1H, CH), 6.792 (s, 1H, =NH), 6.326 (s, 1H, =NH), 4.499 (s, 1H, NH), 4.037- 4.048 (d, 2H, CH_2), 3.311-3.475 (m, 1H, CH), 3.167-3.193 (t, 2H, CH_2), 2.965 (s, 6H, $(CH_3)_2$), 2.605-2.655 (m, 1H, CH), 2.174 (s, 1H, NH,) 2.074-2.086 (t, 2H, CH_2), 1.664-1.678 (m, 2H, CH_2), 1.431-1.447 (t, 3H, CH_3), 1.405-1.422 (d, 6H, $(CH_3)_2$); ^{13}C NMR (DMSO, δ ppm); m/z 512.80 (M^+).

1-(isopropylamino)-3-(naphthalen-1-yloxy)propan-2-yl-3-((N-(N,N-dimethylcarbamimido yl)carbamimidoyl)carbamoyl)-2-propylhex-2-enoate (CO3): Yield: 82%; mp 216°C; FTIR (KBr) cm^{-1}: 3378.87 (N-H stre), 3059.81 (Ar. C-H stre.), 2936.64 (Ali. C-H stre.), 1678.65 (C=O stre.), 1585.60, 1426.27 (Ar C=C stre.), 1290.11 (C-O-C stre.), 1193.47 (C-N); ^{1}H NMR (DMSO, δ ppm) 8.261 (s, 1H, NH), 7.195-8.247 (m, 7H, Ar-H), 6.681 (s, 1H, =NH), 6.484 (s, 1H, C=NH), 4.395 (s, 1H, NH), 4.157-4.160 (d, 2H, CH_2), 3.355-3.369 (m, 1H, CH), 3.001-3.055 (t, 2H, CH_2), 2.916 (s, 6H, $(CH_3)_2$), 2.481-2.499 (m, 1H, CH), 2.069 (s, 1H, NH), 1.812-1.892 (m, 4H, $2CH_2$), 1.513-1.529 (t, 4H, $2CH_2$), 1.266-1.298 (t, 6H, $(CH_3)_2$); 1.096-1.140 (t, 6H, $2CH_3$); ^{13}C NMR (DMSO, δ ppm) 18.187, 18.681, 37.422, 38.374, 38.892, 39.100, 39.309, 39.517, 39.726, 39.935, 40.143, 46.856, 49.856, 65.297, 105.291, 120.243, 121.735, 125.246, 126.152, 126.498, 127.424, 128.492, 130.710, 132.898, 134.012, 153.737, 168.593; m/z 553.50 (M^+ + 1).

1-(isopropylamino)-3-(naphthalen-1-yloxy)propan-2-yl-2-((N'-(N,N-dimethylcarb amimidoyl) carbamimidoyl)carbamoyl)benzoate (CO4):

Yield: 68%; mp 189°C. FTIR (KBr) cm^{-1}: 3361.63 (N-H stre.), 2972.80 (Ar C-H stre.), 2886.59 (Ali C-H stre.), 1684.62 (C=O stre.), 1615.32, 1465.08 (Ar C=C stre.), 1292.01 (C-O-C stre.), 1118.10 (C-N). ^{1}H NMR (DMSO, δ ppm) 8.261 (s, 1H, NH), 7.192-8.243 (m, 11H, Ar-H), 6.653 (s, 1H, =NH), 6.491 (s, 1H =NH), 4.374 (s, 1H, NH), 4.156-4.169 (d, 2H, CH_2), 3.234-3.312 (m, 1H, CH), 3.011-3.058 (t, 2H, CH_2), 2.917 (s, 6H, $(CH_3)_2$), 2.481-2.499 (m, 1H, CH), 2.058 (s, 1H, NH) 1.261-1.291 (d, 6H, $(CH_3)_2$); m/z 519.30 (M^+ + 1).

1-(isopropylamino)-3-(4-(2-methoxyethyl) phenoxy)propan-2-yl-3-((N-(N,N-dimethylcarbaimidoyl)carbamidoyl)carbamoyl-2-Prophylhex-2-enoate (CO5):

Yield: 81%; mp 264°C. FTIR (KBr) cm^{-1}: 3424.87 (N-H stre), 3013.89 (Ar. C-H stre.), 2936.64 (Ali. C-H stre.), 1678.85 (C=O stre.), 1585.60, 1426.27 (Ar C=C stre.), 1290.11 (C-O-C stre.), 1193.47 (C-N); ^{1}H NMR (DMSO, δ ppm) 8.166 (s, 1H, NH), 7.115-7.539 (m, 4H, Ar-H), 6.696 (s, 1H, =NH), 6.434 (s, 1H, =NH), 4.336 (s, 1H, NH), 4.152-4.161 (d, 2H, CH_2), 3.448-3.483 (t, 2H, CH_2), 3.214-3.298 (m, 1H, CH), 3.071-

3.087 (t, 2H, CH_2), 2.922 (s, 6H, $(CH_3)_2$), 2.701-2.736 (t, 2H, CH_2), 2.481-2.499 (m,1H, CH), 2.337 (s, 3H, CH_3), 2.153 (s, 1H, NH), 1.691-1.722 (m, 4H, $2CH_2$), 1.447-1.482 (t, 6H, $2CH_3$), 1.119-1.145 (d, 6H, $(CH_3)_2$); ^{13}C NMR (DMSO, δ ppm) 37.452, 37.617, 38.889, 39.097, 39.305, 39.514, 39.932, 40.140, 132.434, 158.271, 159.103, 166.842; m/z 532.60 (M^+).

1-(isopropylamino)-3-(4-(2-methoxyethyl)phenoxy)propan-2-yl-3-((N-(N,N-dimethylcarbmimidoyl)carbamimidoyl)carbamoyl)-2-propylhex-2-enoate (CO6):

Yield: 75%; mp 168°C. FTIR (KBr) cm^{-1}: 3408.39 (N-H stre), 3018.31 (Ar. C-H stre.), 2889.38 (Ali. C-H stre.), 1682.12 (C=O stre.), 1573.40, 1464.03 (Ar C=C stre.), 1258.42 (C-O-C stre.), 1167.46 (C-N); ^{1}H NMR (DMSO, δ ppm) 8.164 (s, 1H, NH), 7.115-7.539 (m, 4H, Ar-H), 6.696 (s, 1H, =NH), 6.434 (s, 1H, =NH), 4.336 (s, 1H, NH), 4.152-4.161 (d, 2H, CH_2), 3.448-3.483 (t, 2H, CH_2), 3.214-3.298 (m, 1H, CH), 3.043-3.087 (t, 2H, CH_2), 2.923 (s, 6H, $(CH_3)_2$), 2.701-2.736 (t, 2H, CH_2), 2.481-2.499 (m,1H, CH), 2.337 (s, 3H, CH_3), 2.153 (s, 1H, NH), 1.746-1.782 (t, 4H, $2CH_2$), 1.627-1.655 (m, 4H, $2CH_2$), 1.447-1.482 (t, 6H, $2CH_3$), 1.119-1.145 (d, 6H, $(CH_3)_2$); ^{13}C NMR (DMSO, δ ppm) 18.205, 18.638, 37.456, 38.895, 39.103, 39.312, 39.520, 39.729, 39.938, 40.146, 46.916, 49.838, 65.296, 69.973, 105.288, 120.210, 121.750, 124.880, 125.237, 126.151, 126.483, 127.411, 128.384, 130.711, 132.858, 134.008, 153.757, 158.294, 168.602; m/z 559.20 (M^+).

Chemical hydrolysis studies:

Hydrolytic behavior of synthesized co-drugs was studied in Simulated Gastric Fluid (pH 1.2); Simulated Intestinal Fluid (pH 6.8); Simulated Plasma Fluid (pH 7.4). Chemical hydrolysis studies was carried out with USP-II paddle apparatus at a rotational speed of 50 rpm, temperature of 37±1°C, 900 ml solution of pH 1.2, 6.8 and 7.4 were used as dissolution media. 1 ml of the hydrolysis medium was taken out at 0 minute and every 15 min. for 120 min. 1 ml of the pH solution was added to the dissolution vessel[13-14]. The sample withdrawn was analyzed with the HPLC using Phenomenex Luna C_{18} column (250 mm x 4.6 mm id, 5 µm particle size), LC solutions software and mobile phase acetonitrile: water 70:30. Flow rate of mobile phase was kept at 1 mL/min at pressure 120-135 psi and UV detector (SPD-20A with D_2 lamp) was used and retention time and peak area were noted at 226 nm. The comparative study of rate of hydrolysis is shown as follows.

In-silico Evaluation of Physicochemical Properties:

The theoretical study of oral biodisponibility (Lipinski rule-of five) was performed in the Accelrys drug discovery studio. The theoretical oral bioavailability ranking of chemical compounds can be estimated using the Lipinski's 'rule-of-five', since it describes molecular properties important for a drug pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion (ADME). The active compound must present at least three of four rules: H-bond donors (HBD) ≤ 5, H-bond acceptors (HDA) ≤ 10, molecular mass (MM) ≤ 500, and the calculated logP (clogP) ≤ 5[15-18].

Pharmacology:

All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of Acharya & B M Reddy College of Pharmacy, Bengaluru, constituted in accordance with the guidelines of the committee for the purpose of control and supervision of experiment on animals (CPCSEA), Government of India.

IAEC Number: IAEC/ABMRCP/2015-2016/21.

Statistical Analysis: All data obtained from animal experiments were calculated as mean ± SEM. Statistical differences between the synthesized compounds and the control were tested by one-way ANOVA followed by Dunnett's multiple comparison tests.

Level of significance: $P < 0.05$, $P < 0.01$ level, $P<0.0001$.

Animals were divided into five groups. Group-I served as control group (Control-Veh), group-II simultaneous diabetes and renal hypertensive group receiving vehicle (DM + HTN-Veh), group-III simultaneous diabetes and renal hypertensive groups receiving the codrugs 20 mg/kg (DM + HTN- selected codrugs), group IV simultaneous diabetes and renal hypertensive group receiving standard mixture of Propranolol-Metformin. (DM + HTN-PM) and group V simultaneous diabetes and renal hypertensive group receiving standard mixture of Metoprolol-Metformin. (DM + HTN-MM)

Antihypertensive Activity

The blood pressure (BP) was determined with a BIOPAC student, Inc, Tail-cuff method. The restrainer carrying the rat was placed in the BP instrument with tail stand out. The tail was lightly placed in touch with a transducer membrane, which was attached to the digital BP display panel. The instrument was then turned on and allowed to stabilize until steady pulse rate was observed. Then the BP recording button was pressed and the Systolic Blood Pressure was recorded. The test compounds were administered by oral feeding using an oral feeding needle[16-18]. Test compounds (codrugs) were prepared in 0.5% carboxymethyl cellulose (CMC) and dose of 20 mg/kg orally. Prior to dosing the animals, initial graph reading were taken to record the BP. Average readings were calculated by employing ANOVA method.

Antidiabetic Activity

Blood was withdrawn each time from the retro orbital plexus of the eye using capillary tube each time. The blood glucose levels of the animals in each group were checked at 0, 2, 4, 6, 8 and 10 h intervals study using glucometer [17] (Accu-check® active, Germany).

RESULTS AND DISCUSSION

Chemistry

Codrugs of Propranolol and Metoprolol with Metformin were prepared with an intend to enhance the bioavailability of parent drugs and thereby increasing

the duration of action of parent drugs. In the present work phthalic anhydride and substituted succinic, maleic and phthalic anhydride were used as the linkers and the obvious effect that simultaneous delivery of the two drugs as one chemical entity, will have on the pharmacokinetics of each drug. The physicochemical characterization like melting point and spectral characterization by IR, ^{1}H-NMR and mass spectral data were carried out for the synthesized codrugs. All the reactions were monitored using precoated TLC plates. The absence of TLC spots for starting materials and appearance of single new TLC spot at different R_f value ensured completion of the reaction. The TLC plates were visualized either by iodine vapors or by viewing in UV-visible chamber. The reaction product of all the reactions were purified initially by different workup processes to remove unreacted starting materials if any and then recrystallization was carried out. The **co-drugs (CO1-6)** have shown the FTIR spectral data for N-H peak in the range of 3361.63–3424.87 cm^{-1}, Ar. C-H peak in the range of 2972.80-3109.99 cm^{-1}, Ali. C-H peak in the 2886.59-2952.05 cm^{-1}, C=O peak in the range of 1662.88-1703.12 cm^{-1}, C-O-C peak in the range of 1236.59-1298.16 cm^{-1} and C-N peak in the range of 1115.39-1193.47 cm^{-1}.

In the ^{1}H NMR spectra, all protons were seen according to the expected integral values. The ^{1}H NMR of **co-drugs (CO1-6)** have shown the C(O)NH singlet peak in the range of δ 8.162-8.263 ppm, Ar. C-H multiplate peak in the range of δ 7.097-8.247 ppm, two singlet peak for two C=NH group in the range of δ 6. 326-6.792 ppm, two singlet peak for two N-H group in the range of δ 4.334-4.499 ppm & δ 2.058-2.174 ppm, OCH_2 doublet peak in the range of δ 4.037-4.170 ppm, OCH multiplate peak in the range of δ 3.214-3.475 ppm, NCH_2 triplet peak in the range of δ 3.001-3.193 ppm, $N(CH_3)_2$ singlet peak in the range of δ 2.907 to 2.965 ppm, NCH multiplate peak in the range of δ 2.481-2.655 ppm, $CH(CH_3)_2$ peak in the range of δ 1.119-1.422 ppm. Co-drugs were confirmed through the absence of COOH peak of prodrug and the new peak was observed for C(O)NH in the spectra of all the co-drugs.

In the FTIR spectra of all co-drugs, O-H peak of COOH was found to be absent which suggested the formation of co-drugs. FTIR data also shown the lower range of C=O peak of amide which also suggests the formation of co-drugs **(CO1-6)**. The ^{1}H NMR spectrum also supports the scheme of synthesis by the absence of –COOH functional group indicating that it was involved in the reaction. New singlet peak was observed at δ 8.162 - 8.263 ppm which corresponds for the NH of amide group, this confirms the formation of co-drugs, **(CO1-6)** of Propranolol and Metoprolol with Metformin by using various linkers of anhydrides.

Synthesized compounds have shown the respective M^+ peak for CO1, CO2, CO5, CO6, $M^+ + 1$ peak for CO3 and CO4 compound.

In-silico physicochemical properties evaluation:

The various codrugs **(CO1-6)** were submitted to an in silico evaluation using a molecular modeling approach. Since a good absorption after oral administration is obligatory for antihypertensive and antidiabetic activity purpose, we analyzed these compounds according to the rule-of five developed by Lipinski *et al*. **(Table1)**. The rule-of-five theoretically indicates if a chemical compound could be an orally active drug in humans. The rule states that the most 'druglike' molecules present clogP ≤ 5, molecular weight (MW) ≤ 500, and number of hydrogen bond acceptors ≤ 10 and donors ≤ 5. Molecules violating more than one of these rules may have problems with bioavailability. The results showed that all codrugs **(CO1-6) (Table1)** fulfilled the Lipinski 'rule-of-five' as violation of one rule is acceptable.

Table 1. Theoretical oral biodisponibility predicted by using a molecular modeling approach

Codrugs	*Theoretical oral biodisponibility*				
	ALogP	*Mol. Weight*	*No. of Rotatable bonds*	*Hydrogen bond acceptor*	*Hydrogen bond donor*
CO1	4.421	524.65	16	07	05
CO2	3.975	510.62	16	07	05
CO3	5.334	552.70	18	07	05
CO4	3.667	518.60	14	07	05
CO5	3.192	518.64	19	08	05
CO6	4.550	560.72	21	08	05

Chemical Hydrolysis

The Chemical hydrolysis of the synthesized codrugs **(CO1-6)** were studied to determine the stability of codrug at pH 1.2 (non enzymatic Simulated Gastric Fluid, SGF), pH 6.8 (non enzymatic Simulated Intestinal Fluid) whereas potential to generate as the Propranolol, Metoprolol and Metformin at physiological pH 7.4 at 37±5°C using HPLC. The disappearance of the tested compounds and appearance of the peak for standard drug Metformin displayed hydrolysis kinetics over the investigated pH and temperature. The synthesized codrugs showed relative stability in the investigated aqueous solutions and the hydrolysis rates at pH 7.4 are slightly accelerated than

those observed in SGF of pH 1.2 and SIF of pH 6.8. The Hydrolysis pattern of the codrugs at different pH conditions is showed in the **(Figure2)**.

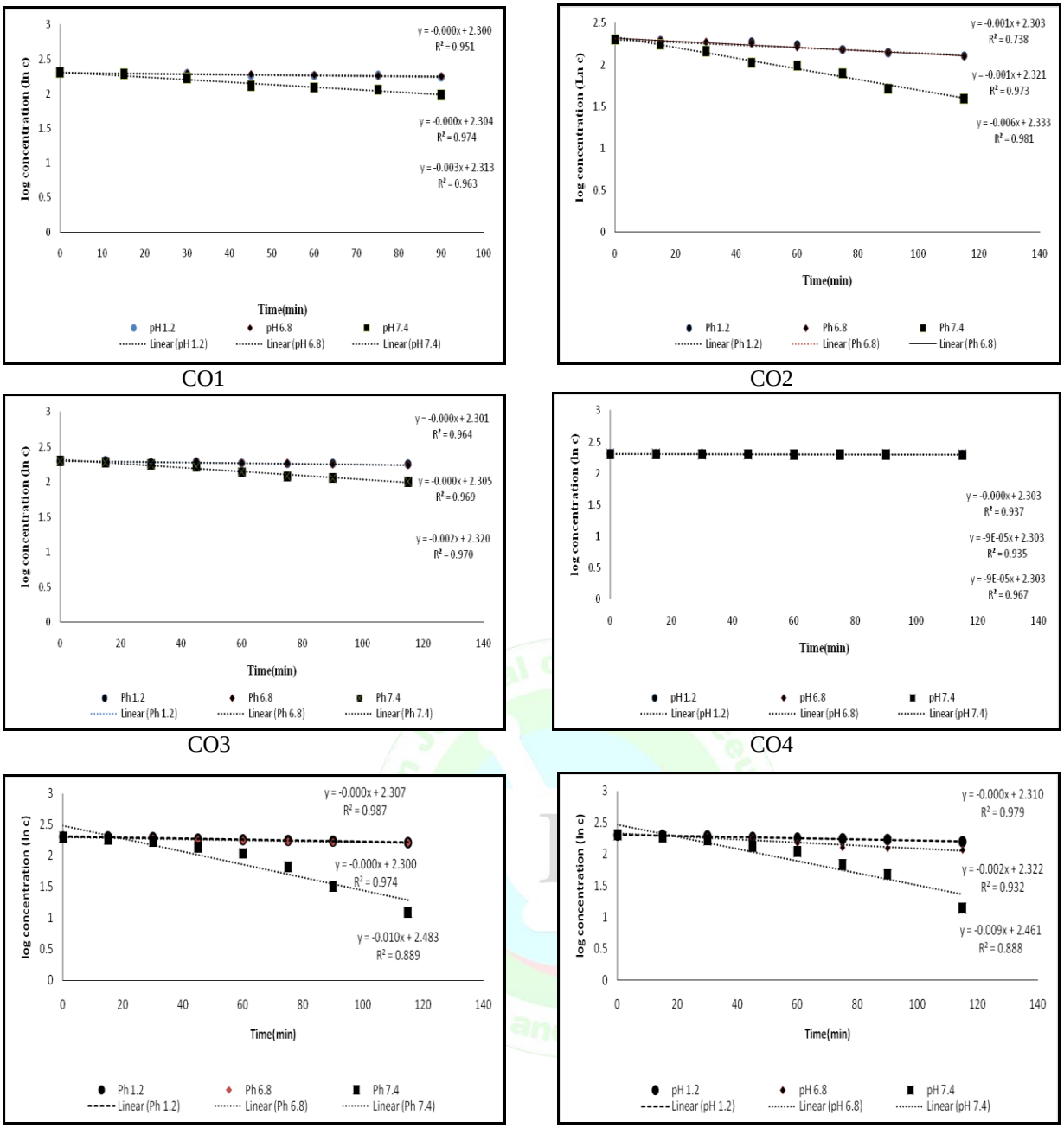


Figure: 2. The hydrolysis rate of codrugs (CO1-6) at pH 1.2, 6.8 and 7.4.

Pharmacological evaluation

The parent drugs Propranolol, Metoprolol and Metformin have been used as a reference substance. All the synthesized codrugs (CO1-6) were screened for their antihypertensive and antihyperglycemic activity by simultaneous induction of two-kidney, one clip renal hypertension and STZ-induced diabetic model in wistar rats.

Antihypertensive activity

The Antihypertensive activity was determined by using Non invasive tail cuff method. After oral administration of standard drugs mixture (20 mg/kg) and synthesized codrugs (20 mg/kg) in rats, the blood pressure was determined from 0 to 12 h. The result showed that all the tested codrugs reduces the blood pressure significantly in comparison with the standard drug Propranolol and Metoprolol. Results of change in blood pressure in experimental study were presented in **Table 2**. The codrugs **CO1** and **CO3** showed faster onset of action and longer duration of action when compared with the standard drug propranolol. Whereas CO3 showed longest duration of action from the Propranolol-Metformin codrug series. All other codrugs

of this series showed longer duration of action but codrug **(CO4)** showed action after long period of time i.e after 900 minutes. In the series of Metoprolol-Metformin codrugs, CO5 showed the faster onset of action compared to that of standard drug Metoprolol. All other codrugs showed almost similar onset of action but duration of action is more when compared to standard drug Metoprolol.

Table 2. Antihypertesive effect of codrugs on simultaneous renal hypertension and STZ-induced diabetic rats.

Groups	*Average Systolic Blood Pressure (mmHg) at time (min)*											
	0	***15***	***30***	***60***	***120***	***180***	***240***	***300***	***360***	***600***	***720***	***900***
Normal	133 ± 0.57	131 ± 0.60	131 ± 0.76	129 ± 0.60	130 ± 0.55	131 ± 0.70	130 ± 0.70	132 ± 0.42	131 ± 0.42	131 ± 0.30	131 ± 0.30	129 ± 0.47
DM+HTN	183 ± 0.73	181 ± 0.42	182 ± 0.47	182 ± 0.49	182 ± 0.60	180 ± 0.66	179 ± 0.60	182 ± 0.49	182 ± 0.42	182 ± 0.54	183 ± 0.60	178 ± 0.60
Prop + Met	182 ± 0.60	180 ± 0.66^{ns}	179 ± 0.66**	166 ± 0.47***	156 ± 0.71***	142 ± 0.88***	134 ± 1.21***	129 ± 1.09***	138 ± 0.87***	147 ± 0.65***	163 ± 0.66***	176 ± 0.57^{ns}
Meto + Met	184 ± 0.36	181 ± 0.42^{ns}	180 ± 0.57^{ns}	154 ± 0.30***	134 ± 0.49***	131 ± 0.47***	141 ± 0.88***	151 ± 0.47***	162 ± 0.61***	170 ± 0.47***	175 ± 0.60***	180 ± 0.42^{ns}
CO1	183 ± 0.47	181 ± 0.42^{ns}	169 ± 0.47^{ns}	155 ± 0.33***	140 ± 0.60***	132 ± 0.96***	131 ± 0.33***	132 ± 0.47***	133 ± 0.33***	134 ± 0.33***	153 ± 0.30***	163 ± 0.61***
CO2	183 ± 0.33	183 ± 0.63^{ns}	181 ± 0.47^{ns}	165 ± 0.60***	153 ± 0.42***	143 ± 0.61***	132 ± 0.63***	133 ± 0.70***	134 ± 0.60***	143 ± 0.56***	150 ± 0.99***	162 ± 0.36***
CO3	181 ± 0.42	179 ± 0.44^{ns}	179 ± 0.49***	152 ± 0.73***	142 ± 0.36***	132 ± 0.42***	131 ± 0.42***	129 ± 0.60***	131 ± 0.68***	133 ± 0.47***	135 ± 0.76***	152 ± 0.36***
CO4	182 ± 0.49	181 ± 0.30^{ns}	181 ± 0.47^{ns}	176 ± 0.47***	174 ± 0.30***	173 ± 0.60***	172 ± 0.42***	169 ± 0.52***	166 ± 0.95***	165 ± 0.30***	163 ± 0.88***	157 ± 0.74***
CO5	182 ± 0.56	180 ± 0.25^{ns}	178 ± 0.30***	143 ± 0.42***	132 ± 0.83***	131 ± 0.42***	132 ± 0.22***	133 ± 0.33***	148 ± 0.79***	165 ± 0.79***	169 ± 0.98***	176 ± 0.73^{ns}
CO6	183 ± 0.47	180 ± 0.57	177 ± 0.30^{ns}	148 ± 0.68***	133 ± 0.40***	133 ± 0.72***	129 ± 0.73***	132 ± 0.99***	134 ± 0.55***	148 ± 0.70***	164 ± 0.79***	172 ± 0.49***

Antidiabetic Activity

After oral administration of standard drugs mixture (20 mg/kg) and synthesized codrugs (20 mg/kg) in rats, the blood glucose was determined from 0 to 10 h. The result showed that all the codrugs reduces the blood glucose significantly as compared to standard drugs mixture. Results of change in blood glucose in experimental study were presented in Table 3. Codrugs CO2 (124 ± 0.7638), found to caused lowering in blood glucose level than that of standard drugs mixture of Propranolol-Metformin (160 ± 0.6191) and Metoprolol-Metformin (167 ± 1.208). Codrug (CO7) (335 ± 0.7149) didn't decrease the blood glucose level.

Table 3. Antihyperglycemic effect of codrugs on simultaneous renal hypertension and STZ-induced diabetic rats.

Groups	*Blood glucose level (mg/dl) at time (min)*					
	0	***120***	***240***	***360***	***480***	***600***
Normal	86 ± 0.6708	85 ± 0.5774	84 ± 0.6009	82 ± 0.3073	83 ± 0.4282	84 ± 0.4282
DM+HTN	333 ± 0.8602	333 ± 7071	332 ± 0.7746	333 ± 0.6000	334 ± 0.9487	336 ± 1.049
Prop + Met	338 ± 0.763	268 ± 0.8119***	223 ± 0.7638***	210 ± 0.8851***	180 ± 0.8028***	160 ± 0.6191***
Meto + Met	339 ± 1.685	267 ± 0.8602***	228 ± 0.9274***	190 ± 0.5831***	175 ± 1.208***	167 ± 1.208***
CO1	357 ± 0.666	315 ± 1.335***	288 ± 1.116***	255 ± 0.9545***	206 ± 0.9458***	183 ± 0.7601***
CO2	362 ± 0.666	253 ± 0.542***	235 ± 0.3416***	179 ± 0.9574***	136 ± 1.155***	124 ± 0.7638***
CO3	357 ± 0.4216	343 ± 0.8724^{ns}	333 ± 1.155^{ns}	308 ± 1.838***	285 ± 1.155***	265 ± 0.6667***
CO4	352 ± 0.7303	351 ± 0.6667^{ns}	352 ± 0.4216***	346 ± 0.7638***	339 ± 0.7032**	335 ± 0.7149^{ns}
CO5	365 ± 1.116	326 ± 0.9545^{ns}	298 ± 0.9458***	288 ± 1.302***	252 ± 0.7601***	203 ± 0.9661***
CO6	357 ± 0.9916	344 ± 1.302^{ns}	333 ± 0.9458^{ns}	312 ± 0.8819***	286 ± 0.7678***	264 ± 1.054***

CONCLUSION

The synthesis of codrugs of Propranolol and Metoprolol with Metformin was successfully effected in a rather

simple and scalable scheme that consist of two steps. The chemical structures of the codrug and the intermediate were confirmed by FT-IR, ^{1}H NMR, and MS analysis. Absorption bands obtained in IR and NMR spectrum confirmed the formation of amide linkage between Propranolol and Metoprolol with Metformin. Preliminary kinetic study for compounds **CO1-6** revealed that compounds are chemically stable to a great extent at pH 1.2 and pH 6.8. While they shows chemical hydrolysis at pH 7.4. On the basis of chemical hydrolysis studies and pharmacological evaluation it suggests that the linkers like propyl maleate and dipropyl maleate, act as a good linkers, as codrugs containing this linkers are showing the slower hydrolysis at the pH 7.4 and having the longer duration of action. The codrugs containing phthalate as a linker get hydrolyzed in a very negligible amount hence cannot get converted in to the parent drugs and it also didn't displayed any pharmacological activity, so phthalate act as the very poor linkers in the codrug design. It was found that more the number of carbons in the linkage, chemical hydrolysis was slow and if the numbers of carbons are less in the linkage chemical hydrolysis were faster. Hydrolysis pattern of the best codrug indicate the release of the active drugs for longer period of time at pH 7.4 and no hydrolysis at pH 1.2 and 6.8. pH specific hydrolysis and slower hydrolysis of certain codrugs indicates the rate-controlled and time controlled drug delivery of the actives.

ACKNOWLEDGMENTS

The authors are thankful to the Principal Dr. Divakar Goli and Shri. Premnath Reddy, Chairman of Acharya Institutes, Bangalore, for providing laboratory facilities. Authors express their thanks to M/s. Cadila Healthcare Limited, Ahmadabad (India) and Strides Pharma Sciences Ltd. (India). (India) for providing gift samples of atenolol and metformin. Authors are also grateful to Indian Institute of Sciences, Bangalore, for providing spectral data.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Black J. Reflections on drug research. Br J Pharmacol 2010; 161:1204-1216.
2. Akbar S, Alorainy MS. The current status of beta blockers' use in the management of hypertension. Saudi Med J 2014; 35(11):1307-1317.
3. Dandona P, Fonseca V, Mier A, Beckett AG. Diarrhea and Metformin in a Diabetic Clinic. Diabetes Care 1983; 6:472–474.
4. Krentz AJ, Ferner RE, Bailey CJ. Comparative Tolerability Profiles of Oral Antidiabetic Agents. Drug Saf 1994; 11:223–241.
5. Garber AJ, Duncan TG, Goodman AM, Mills DJ, Rohlf JL. Efficacy of Metformin in Type II Diabetes: Results of a DoubleBlind,
6. Placebo-Controlled, Dose-Response Trial. Am J Med 1997; 103:491–497.
7. Belcher G, Lambert C, Edwards G, Urquhart R, Matthews DR. Safety and Tolerability of Pioglitazone, Metformin, and Gliclazide in the Treatmentof Type2 Diabetes. Diabetes Res Clin Pract 2005; 70:53–62.
8. Sowers JR, Zemel MB. Clinical implications of hypertension in the diabetic patient: A Review. Am J Hypertens 1990; 3:415-424.
9. Okosun IS Chandra KM, Choi S, Cristman J, Dever GE, Prewitt TE. Hypertension and type 2 diabetes comorbidity in adults in the United States: risk of overall and regional adiposity. Obes Res 2001; 9(1):1-9.
10. Reaven GM, Hoffman BB. Symposium on diabetes and hypertension. Am J Med 1989; 87:1S-42S.
11. Kankanalaa K, Billurb R, Reddy VR, Mukkantia K, Palb S. TFAA-H_3PO_4 mediated rapid and single-step synthesis of mutual prodrugs of paracetamol and NSAIDs. Green Chem Lett Reviews 2012; 5(3):421-432.
12. Andhale GS, Giles D, Das AK, Bose A, Metikurki B. Design, Synthesis and Chemical Hydrolysis study of codrugs of propranolol with metformin. Eur J Pharma Med Res 2018; 5:395-404.
13. Andhale GS, Giles D, Das AK, Metikurki B, Gurubasavarajaswamy PM. Design, Synthesis and Chemical Hydrolysis study of codrugs of metoprolol with metformin. Inter J Pharma Sci Drug Res 2018; 10:95-102.
14. Baidya M, Das AK. Synthesis and Hydrolytic Kinetic study of some Antihypertensive Co-drugs. Indian J Hetero Chem 2011; 20:343-346.
15. Nielsenw NW, Bundgaard H. Glycolamide esters as biolabile prodrugs of carboxylic acid agents: synthesis, stability, bioconversion, and physicochemical properties. J Pharm Sci 1988; 77:285–298.
16. Alam O, Khan SA, Siddiqui N, Ahsan W, Verma SP, Gilani SJ. Antihypertensive activity of newer 1,4-dihydro-5-pyrimidine carboxamides: Synthesis and pharmacological evaluation. Eur J Med Chem 2010; 45:5113-5119.
17. Bhutani R, Pathak DP, Kaoor G, Husain A, Kant R, Iqbal MA. Synthesis, molecular modeling studies and ADME prediction of benzothiazole clubbed oxadiazole-mannich bases, and evaluation of their anti-diabetic activity through in vivo model. Bioorg Chem 2018; 77:6-15.
18. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 2001; 46(1-3):3-26.