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Design, Synthesis and Chemical Hydrolysis Study of Codrugs of Metoprolol with Metformin

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ABSTRACT

In the present study we have synthesized ester prodrugs (Pa-i) of Metoprolol (I) by using pthalic anhydride and derivatives of succinic and maleic anhydride. Different codrugs (COa-i) were synthesized from prodrugs of metoprolol. All the codrugs were characterized by melting point, FTIR, NMR and Mass Spectroscopy. The chemical hydrolysis of COa-i were investigated at the pH 1.2, 6.8 and 7.4. Presence of maleate, methyl maleate, dimethyl maleate and succinate group as linker were found to possess good hydrolysis when compared to that of other substitutes. Among the synthesized codrugs, COf is found to be the best one among the series.

Keywords: Chemical hydrolysis, codrugs, metformin, metoprolol, prodrugs.

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INTRODUCTION

Metoprolol, a β selective adrenergic blocking agent is a well-established drug in the handling of angina pectoris, myocardial infarction and congestive heart failure as well as hypertension. Metoprolol reduces blood pressure by decreasing of cardiac output via slowing of the heart rate and is useful as first line therapy in the treatment of mild to moderate essential hypertension. However, the efficacy of the drug is reduced by its extensive first pass metabolism following oral administration. [1-2]

Metformin, N,N-dimethylimidodicarbonimidicdiamide is a potent insulin-sensitizing biguanidine used to treat type 2 diabetes and usually considered to be a first-line treatment, particularly in obese and/or hyperlipidemic NIDDM patients. The antihyperglycemic effects of metformin are not only the inhibition of intestinal glucose absorption and the improvement of peripheral

and hepatic insulin sensitivity but also the reduction of hepatic glucose production and the enhancement of peripheral glucose utilization, although the exact mechanism of action is still uncertain. [3-5] In addition, metformin has favorable effects on dyslipidemia [6], hypertension [7], vascular function [8] and fibrinolytic activity [9], which are very beneficial to patients with NIDDM, the major risk factor group of atherosclerosis or cardiovascular disease. Unfortunately, this effective but highly basic antihyperglycemic agent is fully protonated under physiological conditions and therefore slowly and incompletely absorbed from the upper intestine after oral administration. Together with a rapid kidney excretion [10], metformin suffers from variable bioavailability and causes uncomfortable gastrointestinal adverse effects at effective doses (0.5-2 g per day), such as abdominal discomfort and pain, nausea, vomiting, diarrhea, anorexia, and metallic taste, for nearly 30% of patients. [11-14]

The prodrug approach is usually used to improve physiological, biopharmaceutical, and drug delivery properties of therapeutic agents. Ideally, an inactive pro-moiety is covalently attached to the parent molecule, and the resulting prodrug is converted to the parent drug in the body prior to it exhibits its pharmacological effect. [15] A codrug or mutual prodrug, consists of two different synergistic drugs within a single chemical entity. The two drugs may be connected either directly or by means of cleavable covalent linker. Many 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. [16]

Elevated blood pressure is known to contribute to diabetic microvascular and macrovascular complications. Fortunately, reductions in blood pressure can decrease the risk of these complications. Beta-blocker therapy can be advantageous in many patients with diabetes because of its proven ability to decrease cardiovascular morbidity and mortality in persons with atherosclerotic heart disease.

Codrug or mutual prodrug is an approach where various effective drugs, which are associated with some drawbacks, can be modified by attaching with other drugs of same or different categories directly or via a linkage. More appropriately one can say combining two different pharmacophores with similar or different pharmacological activities elicit synergistic action or help to target the parent drug to specific site/organ/cells respectively. This approach is commonly used to improve physicochemical, biopharmaceutical and drug delivery properties of therapeutic agents. [17]

Combination drug therapy is used now a day, to limit the side effects of the therapeutic agents, used alone in the treatment of certain diseases, with complex and heterogeneous pathogenesis. This can be achieved by concomitant administration of two or more single active drugs or by designing hybrid molecules. These hybrid molecules often consist of different pharmacophore, which are linked to each other via spacers. Hypertension and diabetes frequently occur together. Framingham's study showed that about 58% of the subjects did have hypertension at the time that they were diagnosed with diabetes mellitus. Coexistence of diabetes and hypertension in human is associated with higher cardiovascular risk and mortality. [18] In this regard we have planned to prepare and study the chemical hydrolysis of codrugs of antihypertensive drug metoprolol and antidiabetic drug metformin.

MATERIALS AND METHODS

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 ^1H NMR spectra were recorded on a Bruker (300 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.

Synthetic studies

Synthesis of prodrugs of Metoprolol (Pa-i)

Metoprolol (I) (1.69 mmoles) was stirred at 85-90°C with different anhydrides of dicarboxylic acid (5.00 mmoles) and dimethyl formamide (DMF) (1 ml) for a period of 12-15 h. The mixture was then cooled to room temperature, water (10 ml) was added and the solution was washed with ether to remove DMF and the amide derivatives. The aqueous solution was evaporated to yield Pa-i. [19] The schematic representation of synthesis of Pa-i by using various anhydrides dicarboxylic acid is shown in "Figure 1".

Synthesis of codrugs of metoprolol prodrugs with metformin (COa-i)

A two-necked round bottom flask was equipped with a dean-stark apparatus topped with a reflux condenser and a nitrogen inlet. Metoprolol prodrugs (6 mmoles), boric acid (1 g) and methanol (20 ml) were kept in the reaction vessel. To this stirred colorless mixture metformin (8.2 mmoles) was added in portions. The reaction mixture was heated at reflux for 16-18 h and

water was collected in the dean-stark trap. The mixture was then poured into dichloromethane (10 ml) which led to the formation of precipitate. The obtained product was then dried in vacuum. [20] The schematic representation of synthesis of COa-i is shown in Figure 1.

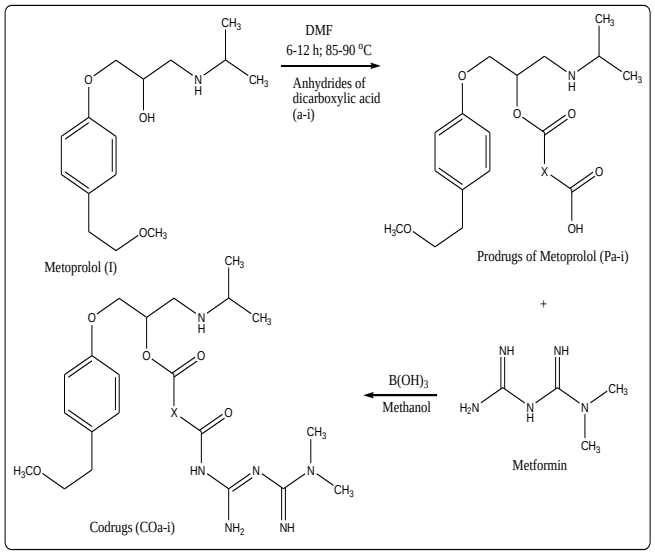
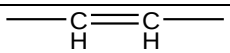
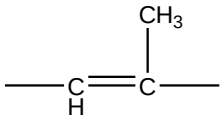
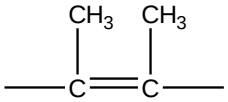
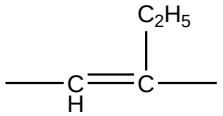
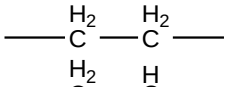
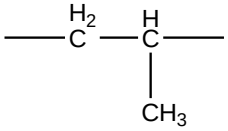
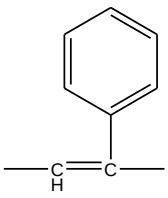
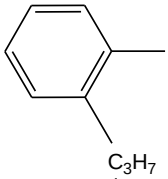
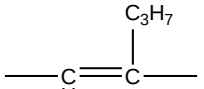
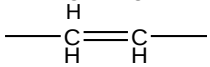
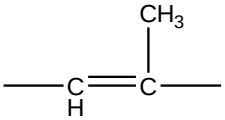
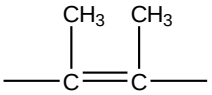
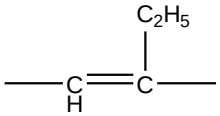
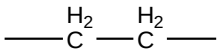
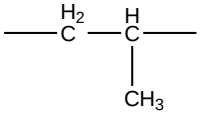
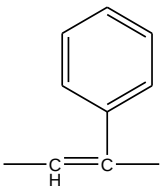
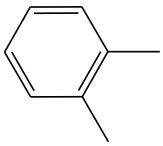
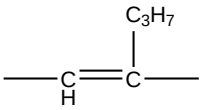


Fig. 1: Schematic representation of synthesis of metoprolol prodrugs (Pa-i) and codrugs (COa-i).

S. No.	Comp. Code	X
1	Pa	
2	Pb	
3	Pc	
4	Pd	
5	Pe	
6	Pf	
7	Pg	
8	Ph	
9	Pi	
10	COa	
11	COb	
12	COc	
13	COd	
14	COe	
15	COf	
16	COg	
17	COh	
18	COi	

Physicochemical and spectral characterization of (Pa-i) and (COa-i)

3-((3-(4-(2-methoxyethyl)phenoxy)-1-(isopropylamino) propan-2-yloxy)carbonyl)acrylic acid (Pa)

Yield: 74.25 %; mp 255°C. FTIR (KBr) cm^{-1}: 3389.28 (N-H stre.), 3122.36 (O-H stre.), 3019.51 (Ar. C-H stre.), 2936.41 (Ali. C-H stre.), 1739.21 (C=O stre), 1552.29 and 1441.28 (Ar. C=C stre.), 1127.10 (C-O-C stre.). ^{1}H NMR (DMSO, δ ppm) 13.457 (s, COOH), 7.154-7.547 (m, 4H, Ar-H), 6.532-6.550 (d, C(O)CH), 6.162-6.349 (d, C(O)CH), 4.268-4.299 (m, 1H, OCH), 3.826 (s, 3H, OCH_3), 3.538-3.547 (d, 2H, OCH_2), 3.337-3.376 (t, 2H, CH_2O), 3.111 (s, 1H, NH), 2.978-3.153 (t, 2H, NCH_2), 2.672-2.694 (m, 1H, NCH), 2.125-2.238 (t, 2H, C-CH_2-C), 2.213-2.349 (d, 6H, $(CH_3)_2$). m/z 365 (M^+).

3-((3-(4-(2-methoxyethyl)phenoxy)-1-(isopropylamino) propan-2-yloxy)carbonyl)-2-methylacrylic acid (Pb)

Yield: 91.66%; mp 203°C. FTIR (KBr) cm^{-1}: 3334.85 (N-H stre.), 3112.69 (O-H stre.), 3074.79 (Ar. C-H stre.), 2925.61 (Ali C-H stre.), 1769.20 (C=O stre), 1584.22 and 1450.34 (Ar. C=C stre), 1132.25 (C-O-C stre.). ^{1}H NMR (DMSO, δ ppm) 13.578 (s, 1H, COOH), 6.979-7.532 (m, 4H, Ar-H), 6.550 (s, C(O)CH), 4.153-4.203 (m, 1H, OCH), 3.970 (s, 3H, OCH_3), 3.715-3.725 (d, 2H, OCH_2), 3.376-3.394 (t, 2H, CH_2O), 3.553 (s, 1H, NH), 2.800-3.832 (t, 2H, NCH_2), 2.523-2.621 (m, 1H, NCH), 2.175-2.293 (t, 2H, C-CH_2-C), 1.633 (s, 3H, =C(CH_3), 1.241-1.293 (d, 6H, $(CH_3)_2$). m/z 381 (M^+).

3-((3-(4-(2-methoxyethyl)phenoxy)-1-(isopropylamino) propan-2-yloxy)carbonyl)-2-methylbut-2-enoic acid (Pc)

Yield: 79.56%; mp 158°C. FTIR (KBr) cm^{-1}: 3350.30 (N-H stre.), 3114.37 (O-H stre.), 3088.50 (Ar. C-H stre.), 2889.14 (Ali. C-H stre.), 1772.25 (C=O stre.), 1577.22 and 1475.72 (Ar. C=C stre.), 1079.58 (C-O-C stre.). ^1H NMR (DMSO, δ ppm) 13.409 (s, COOH), 7.162-7.435 (m, 4H, Ar-H), 4.153-4.203 (m, 1H, OCH), 3.979 (s, 3H, OCH_3), 3.745-3.759 (d, 2H, OCH_2), 3.393-3.401 (t, 2H, CH_2O), 3.527 (s, 1H, NH), 2.826-2.845 (t, 2H, NCH_2), 2.680-694 (m, 1H, NCH), 2.299-2.393 (t, 2H, C-CH_2-C), 2.167 (s, 6H, =$C(CH_3)_2$), 1. 377-1.409 (d, 6H, $(CH_3)_2$). m/z 396 (M^+).

2-(((3-(4-(2-methoxyethyl)phenoxy)-1-(isopropylamino)propan-2-yloxy)carbonyl)methylene) butanoic acid (Pd)

Yield: 89.18%; mp 213°C. FTIR (KBr) cm^{-1}: 3400.59 (N-H stre.), 3210.88 (O-H stre.), 3090.36 (Ar. C-H stre.), 2925.43 (Ali C-H stre.), 1746.53 (C=O stre), 1588.35 and 1470.81 (Ar. C=C stre.), 1235.45 (C-O-C stre.). ^1H NMR (DMSO, δ ppm) 13.578 (s, COOH), 6.826-7.377 (m, 4H, Ar-H), 6.578 (s, C(O)CH), 4.162 (s, 3H, OCH_3), 3.826-3.832 (m, 1H, OCH), 3.341-3.358 (t, 2H, CH_2O), 3.559 (s, 1H, NH), 3.377-3.376 (d, 2H, OCH_2), 2.889-2.920 (t, 2H, NCH_2), 2.527-2.567 (m, 1H, NCH), 1.899-1.937 (t, 2H, C-CH_2-C), 1.680-1.694 (m, 2H, CH_2CH_3), 1.523-1.532 (t, 3H, CH_2CH_3)), 1.155-1.213 (d, 6H, $(CH_3)_2$). m/z 396 (M^+).

2-(((3-(4-(2-methoxyethyl)phenoxy)-1-(isopropylamino)propan-2-yloxy)carbonyl) methylene)pentanoic acid (Pe)

Yield: 81.81%; 234°C. FTIR (KBr) cm^{-1}: 3390.12 (N-H stre.), 3165.90 (O-H stre.), 3080.45 (Ar. C-H stre), 2920.45 (Ali. C-H stre.), 1740.60 (C=O stre.), 1680.45 and 1465.38 (Ar. C=C stre.), 1120.30 (C-O-C stre.). ^1H NMR (DMSO, δ ppm) 13.733 (s, COOH), 6.924-7.269 (m, 4H, Ar-H), 4.268-4.299 (m, 1H, OCH), 3.803 (s, 3H, OCH_3), 3.536-3.544 (t, 2H, CH_2O), 3.434 (s, 1H, NH), 3.393-3.409 (d, 2H, OCH_2), 3.162-3.216 (t, 2H, NCH_2), 2.578-2.680 (t, 2H, $NC(O)CH_2$), 2.389-2.425 (m, 1H, NCH), 2.236-2.341 (t, 2H, C-CH_2-C), 1.715-1.748 (t, 2H, $OC(O)CH_2$), 1.541-1.553 (t, 2H, $OC(O)CH_2$), 1.203-1.310 (d, 6H, $(CH_3)_2$). m/z 367 (M^+).

3-((3-(4-(2-methoxyethyl)phenoxy)-1-(isopropylamino)propan-2-yloxy)carbonyl)-2-phenylacrylic acid (Pf)

Yield: 85.79%; mp 226°C. FTIR (KBr) cm^{-1}: 3355.25 (N-H stre.), 3111.89 (O-H stre.), 3080.41 (Ar. C-H stre.), 2920.45 (Ali. C-H stre.), 1759.39 (C=O stre.), 1589.14 and 1465.22 (Ar. C=C stre.), 1081.25 (C-O-C stre.). ^1H NMR (DMSO, δ ppm) 12.832 (s, COOH), 6.924-7.351 (m, 4H, Ar-H), 4.300-4.316 (m, 1H, OCH), 4.203-4.351 (d, 2H, OCH_2), 3.968 (s, 3H, OCH_3), 3.501-3.536 (t, 2H, CH_2O), 3.384 (s, 1H, NH), 3.155-3.213 (m, 1H, NC(O)CH), 2.970-2.979 (t, 2H, NCH_2), 2.794-2.803 (m, 1H, NCH), 2.541-2.559 (d, 2H $OC(O)CH_2$), 2.189-2.243 (t, 2H, C-CH_2-C), 1.536-1.544 (d, 3H, C-CH_3), 1.122-1.138 (d, 6H, $(CH_3)_2$). m/z 381 (M^+).

3-((3-(4-(2-methoxyethyl)phenoxy)-1-(isopropylamino) propan-2-yloxy)carbonyl)propanoic acid (Pg)

Yield: 75.00%; mp 219°C. FTIR (KBr) cm^{-1}: 3355.20 (N-H stre.), 3159.31 (O-H stre.), 3094.58 (Ar. C-H stre.), 2965.06 (Ali. C-H stre.), 1759.35 (C=O stre.), 1621.10 and 1474.50 (Ar. C=C stre.), 1240.11 (C-O-C stre.). ^1H NMR (DMSO, δ ppm) 12.985 (s, COOH), 7.535-8.184 (m, 5H, Ar-H), 6.614-7.177 m, 4H, Ar-H), 6.441 (s, C(O)CH), 4.105-4.211 (m, 1H, OCH), 3.971(s, 3H, OCH_3), 3.527 (s, 1H, NH), 3.337-3.376 (t, 2H, CH_2O), 3.122-3.213 (d, 2H, OCH_2), 2.889-2.920 (q, 2H, NCH_2), 2.680-2.694 (m, 1H, NCH), 2.158-1.211 (t, 2H, C-CH_2-C), 1.102-1.212 (d, 6H, $(CH_3)_2$). m/z 442 (M^+).

2-(((3-(4-(2-methoxyethyl)phenoxy)-1-(isopropylamino)propan-2-yloxy)carbonyl)methyl) propanoic acid (Ph)

Yield: 77.59%, mp 205°C. FTIR (KBr) cm^{-1}: 3360.75 (N-H stre.), 3080.41 (Ar C-H stre.), 2925.75 (Ali C-H), 2840.70 (O-H stre.), 1750.45 (C=O stre.), 1587.80 and 1460.30 (Ar C=C stre.), 1124.35 (C-O-C stre.). ^1H NMR (DMSO, δ ppm) 13.849 (s, COOH), 8.166-8.269 (m, 4H, Ar-H), 7.384-7.849 (m, 7H, Ar-H), 3.832 (s, 3H, OCH_3), 3.376-3.389 (t, 2H, CH_2O), 3.536 (s, 1H, NH), 3.377-3.441 (m, 1H, OCH), 3.268-3.299 (d, 2H, OCH_2), 3.113-3.167 (t, 2H, NCH_2), 2.523-2.553 (m, 1H, NCH), 2.235-2.337 (t, 2H, C-CH_2-C), 1.300-1.326 (d, 6H, $(CH_3)_2$). m/z 416 (M^+).

2-((3-(4-(2-methoxyethyl)phenoxy)-1-(isopropylamino) propan-2-yloxy)carbonyl)benzoic acid (Pi)

Yield: 79.64%, mp 267°C. FTIR (KBr) cm^{-1}: 3390.46 (N-H stre.), 3215.92 (O-H stre.), 3075.25 (Ar. C-H stre.), 2910.40 (Ali C-H stre.), 1741.51 (C=O stre), 1590.35 and 1465.81 (Ar. C=C stre.), 1226.45 (C-O-C stre.). ^1H NMR (DMSO, δ ppm) 13.694 (s, COOH), 6.826-7.377 (m, 4H, Ar-H), 6.687 (s, C(O)CH), 4.262 (s, 3H, OCH_3), 3.826-3.832 (m, 1H, OCH), 3.341-3.358 (t, 2H, CH_2O), 3.559 (s, 1H, NH), 3.377-3.376 (d, 2H, OCH_2), 2.889-2.920 (t, 2H, NCH_2), 2.578-2.623 (m, 1H, NCH), 1.899-1.937 (t, 2H, C-CH_2-C), 1.680-1.694 (m, 2H, **CH_2**CH_2CH_3), 1.523-1.532 (m, 2H, **CH_2**CH_3)), 1.376-1.395 (t, 2H, CH_2CH_2**CH_3**), 1.155-1.213 (d, 6H, $(CH_3)_2$). m/z 396 (M^+).

1-(isopropylamino)-3-(4-(2-methoxyethyl)phenoxy) propan-2-yl-4-((E)-2-(N,N-dimethylcarbamimidoyl) guanidino)-4-oxobut-2-enoate (COa)

Yield: 84.94%, mp 273°C. FTIR (KBr) cm^{-1}: 3374.22 (N-H stre), 3025.27 (Ar C-H stre.), 2940.50 (Ali. C-H stre.), 1732.69 (C=O stre.), 1665.55 (C=N stre.), 1562.36 & 1458.78 (Ar C=C stre.), 1145.12 (C-O-C stre.), 1055.14 (C-N). ^1H NMR (DMSO, δ ppm) 8.316 (s, 1H, C(O)NH), 7.525 (s, 2H, NH_2), 6.868-7.351 (m, 4H, Ar-H), 6.441 (s, 1H, C=NH), 6.525-6.551 (d, C(O)CH), 6.273-6.349 (d, C(O)CH), 4.153-4.203 (m, 1H, OCH), 3.979 (s, 3H, OCH_3), 3.745-3.759 (d, 2H, OCH_2), 3.541-2.553 (t, 2H, CH_2O), 3.441 (s, 1H, NH), 2.745-2.759 (t, 2H, NCH_2), 2.525-2.551 (m, 1H, NCH), 2.441 (s, 6H, $N(CH_3)_2$), 2.163-2.241 (t, 2H, C-CH_2-C), 1.222-1.338 (d, 6H, $(CH_3)_2$). m/z 477 (M^+).

1-(isopropylamino)-3-(4-(2-methoxyethyl)phenoxy) propan-2-yl-4-((E)-2-(N,N-dimethyl carbamimidoyl) guanidino)-3-methyl-4-oxobut-2-enoate (COb)

Yield: 75.19%; mp 202°C. FTIR (KBr) cm^{-1}: 3357.12 (N-H stre.), 3068.20 (Ar. C-H stre.), 2930.48 (Ali. C-H stre.), 1690.22 (C=O stre.), 1640.23 (C=N Stre.), 1588.35 & 1445.40 (Aromatic C=C stre.), 1222.35 (C-O-C stre.), 1075.38 (C-N). ^{1}H NMR (DMSO, δ ppm) 8.144 (s, 1H, C(O)NH), 7.715 (s, 2H, NH_2), 6.629-7.848 (m, 4H, Ar-H), 6.351 (s, 1H, C=NH), 6.203 (s, C(O)CH), 4.164-4.203 (m, 1H, OCH), 4.093 (s, 3H, OCH_3), 3.745-3.759 (d, 2H, OCH_2), 3.542-3.647 (t, 2H, CH_2O), 3.389 (s, 1H, NH), 2.831-2.845 (t, 2H, NCH_2), 2.536-2.501 (m,1H, NCH), 2.425 (s, 6H, $N(CH_3)_2$), 2.129-2.185 (t, 2H, C-CH_2-C), 1.542 (s, 3H, $C(CH_3)$,1.057-1.099 (d, 6H, $(CH_3)_2$). m/z 493 (M^+).

1-(isopropylamino)-3-(4-(2-methoxyethyl)phenoxy) propan-2-yl-4-(-2-(N,N-dimethyl carbamimidoyl) guanidino)-2,3-dimethyl-4-oxobut-2-enoate (COc)

Yield: 82.03%; mp 235°C. FTIR (KBr) cm^{-1}: 3364.51 (N-H stre.), 3079.89 (Ar C-H stre.), 2900.85 (Ali C-H stre.), 1695.32 (C=O stre.), 1655.80 (C=N stre.), 1594.35 & 1468.60 (Ar C=C stre.), 1263.33 (C-O-C stre.), 1168.15 (C-N stre.). ^{1}H NMR (DMSO, δ ppm) 8.159 (s, 1H, C(O)NH), 7.434 (s, 2H, NH_2), 6.759-7.125 (m, 4H, Ar-H), 6.425 (s, 1H, C=NH), 4.538-4.547 (m, 1H, OCH), 4.162(s, 3H, OCH_3), 3.715 (s, 1H, NH), 3.629-3.543 (d, 2H, OCH_2), 3. 423-3.453 (t, 2H, CH_2O), 2.541-2.553 (m, 1H, NCH), 2.451-2.659 (t, 2H, NCH_2), 2.326 (s, 6H, $N(CH_3)_2$), 2.145-2.236 (t, 2H, C-CH_2-C), 1.762 (s, 6H, =$C(CH_3)_2$), 1.300-1.326 (d, 6H, $(CH_3)_2$). m/z 507 (M^+).

1-(isopropylamino)-3-(4-(2-methoxyethyl)phenoxy) propan-2-yl-3-((-N'-(N,N-dimethyl carbamimidoyl) carbamimidoyl)carbamoyl)pent-2-enoate (COd)

Yield: 76.56%; mp 188°C. FTIR (KBr) cm^{-1}: 3389.16 (N-H stre.), 3073.28 (Ar. C-H stre.), 2937.25 (Ali C-H stre.), 1710.40 (C=O stre.), 1666.26 (C=N stre.), 1595.30 & 1460.85 (Ar C=C stre.), 1231.68 (C-O-C stre.), 1180.408 (C-N stre.). ^{1}H NMR (DMSO, δ ppm) 8.435 (s, 2H, C(O)NH), 7.739 (s, 2H, NH_2), 6.766-7.535 (m, 4H, Ar-H), 6.435 (s, 1H, C=NH), 4.531-4.557 (m, 1H, OCH), 4.140(s, 3H, OCH_3), 3.715-3.722 (d, 2H, OCH_2), 3.377-3.393 (t, 2H, CH_2O), 3.211 (s, 1H, NH), 2.971-3.093 (t, 2H, NCH_2), 2.754-2.868 (m, 1H, NCH), 2.542 (s, 6H, $N(CH_3)_2$), 2.263-2.357 (t, 2H, C-CH_2-C), 1.754-1.868 (m, 2H, =$C(CH_2)$), 1.542-1.647 (t, 3H, $C(CH_3)$),1.162-1.175 (d, 6H, $(CH_3)_2$). m/z 507 (M^+).

1-(isopropylamino)-3-(4-(2-methoxyethyl)phenoxy) propan-2-yl-3-((-N'-(N,N-dimethyl carbamimidoyl) carbamimidoyl)carbamoyl)hex-2-enoate (COe)

Yield: 89.76%; mp 240°C. FTIR (KBr) cm^{-1}: 3390.38 (N-H stre.), 3070.82 (Ar. C-H stre.), 28903.23 (Ali. C-H stre.), 1741.40 (C=O stre.), 1675.29 (C=N stre.), 1585.70 & 1492.98 (Ar C=C stre.), 1235.68 (C-O-C stre.), 1158.40 (C-N stre.). ^{1}H NMR (DMSO, δ ppm) 8.389 (s, 1H, C(O)NH), 7.578 (s, 2H, NH_2), 6.955-7.389 (m, 4H, Ar-H), 6.612 (s, 1H, C=NH), 4.216-4.389 (m, 1H, OCH), 4.133-4.216 (d, 2H, OCH_2), 3.979 (s, 3H, OCH_3), 3.541-3.553 (t, 2H, CH_2O), 3.614 (s, 1H, NH), 2.968-3.099 (t, 2H, NCH_2), 2.745-2.767 (m, 1H, NCH), 2.680-2.694 (t, 2H, $NC(O)CH_2$), 2.541-2.55 (t, 2H, $OC(O)CH_2$), 2.423-2.453 (t, 2H, $OC(O)CH_2$), 2.534 (s, 6H, $N(CH_3)_2$), 1.947-2.185 (t, 2H, C-CH_2-C), 1.139-1.216 (d, 6H, $(CH_3)_2$). m/z 479 (M^+).

1-(isopropylamino)-3-(4-(2-methoxyethyl)phenoxy) propan-2-yl-4-(-2-(N,N-dimethyl carbamimidoyl) guanidino)-4-oxo-3-phenylbut-2-enoate (COf)

Yield: 80.29%; mp 179°C. FTIR (KBr) cm^{-1}: 3365.78 (N-H stre.), 3136.10 (Ar. C-H stre.), 2920.22 (Ali C-H stre.), 1715.34 (C=O stre.), 1680.35 (C=N stre.), 1627.40 & 1480.45 (Ar C=C stre.), 1215.52 (C-O-C stre.), 1218.35 (C-N stre.). ^{1}H NMR (DMSO, δ ppm) 8.337 (s, 1H, C(O)NH), 7.767 (s, 2H, NH_2), 6.941-7.404 (m, 4H, Ar-H), 6.453 (s, 1H, C=NH), 4.337-4.351 (m, 1H, OCH), 4.140-4.143 (d, 2H, OCH_2), 3.845(s, 3H, OCH_3), 3.680-3.694 (t, 2H, CH_2O), 3.393 (s, 1H, NH), 3.000-3.296 (m, 1H, NC(O)CH), 2.889-2.920 (t, 2H, NCH_2), 2.773-2.824 (m, 1H, NCH), 2.441 (s, 6H, $N(CH_3)_2$), 2.532-2.553 (d, 2H $OC(O)CH_2$), 1.837-1.867 (t, 2H, C-CH_2-C), 1.337-1.351 (d, 3H, C-CH_3), 1.113-1.143 (d, 6H, $C(CH_3)_2$). m/z 509 (M^+).

1-(isopropylamino)-3-(4-(2-methoxyethyl)phenoxy) propan-2-yl-4-(2-(N,N-dimethyl carbamimidoyl) guanidino)-4-oxobutanoate (COg)

Yield: 84.94%; mp 273°C. FTIR (KBr) cm^{-1}: 3352.21 (N-H stre.), 3081.26 (Ar C-H stre.), 2938.21 (Ali. C-H stre.), 1695.45 (C=O stre.), 1670.65 (C=N stre), 1623.72 & 1472.92 (Ar C=C stre.), 1154.21 (C-O-C stre.), 1144.74 (C-N stre.). ^{1}H NMR (DMSO, δ ppm) 8.614 (s, 2H, C(O)NH), 7.745-7.985 (m, 5H, Ar-H), 7.496 (s, 2H, -NH_2), 6.754-7.201 (m, 4H, Ar-H), 6.535 (s, 1H, C=NH), 6.496 (s, 1H, CO-(CH)=C), 4.337-4.351 (m, 1H, OCH), 4.113(s, 3H, OCH_3), 3.745-3.753 (d, 2H, OCH_2), 3.551-3.609 (t, 2H, CH_2O), 3.481 (s, 1H, NH), 2.826-2.832 (t, 2H, NCH_2), 2.549-2.753 (m, 1H, NCH), 2.505 (s, 6H, $N(CH_3)_2$), 1.795-2.869 (t, 2H, C-CH_2-C), 1.377-1.393 (d, 6H, $C(CH_3)_2$), m/z 569 (M^+).

1-(isopropylamino)-3-(4-(2-methoxyethyl)phenoxy) propan-2-yl-4-(2-(N,N-dimethyl carbamimidoyl) guanidino)-3-methyl-4-oxobutanoate (COh)

Yield: 82.68.46%; mp 192°C. FTIR (KBr) cm^{-1}: 3371.38 (N-H stre.), 3090.28 (Ar C-H stre.), 2937.44 (Ali C-H stre.), 1725.35 (C=O stre.), 1684.69 (C=N stre.), 1621.81 & 1478.57 (Ar C=C stre.), 1210.80 (C-O-C stre.), 1149.52 (C-N). ^{1}H NMR (DMSO, δ ppm) 8.662 (s, 1H, C(O)NH), 7.672-7.703 (m, 5H, Ar-H), 7.557 (s, 2H, NH_2), 6.832-7.184 (m, 4H, Ar-H), 6.629 (s, 1H, C=NH), 4.541-4.550 (m, 1H, OCH), 4.125 (s, 3H, OCH_3), 3.753-3.759 (d, 2H, OCH_2), 3.635-3.691 (t, 2H, CH_2O), 3.557 (s, 1H, NH), 2. 849-2.979 (t, 2H, NCH_2), 2.753-2.767 (m, 1H, NCH), 2.541 (s, 6H, $N(CH_3)_2$), 2.816-2.889 (t, 2H, C-CH_2-C), 1.216-1.389 (d, 6H, $C(CH_3)_2$). m/z 527 (M^+).

1-(isopropylamino)-3-(4-(2-methoxyethyl)phenoxy) propan-2-yl-2-((N'-(N,N-dimethyl carbamimidoyl) carbamimidoyl)carbamoyl)benzoate (COi)

Yield: 69.42%; mp 198°C. FTIR (KBr) cm^{-1}: 3385.25 (N-H stre.), 3075.50 (Ar. C-H stre.), 2914.21 (Ali C-H stre.), 1726.34 (C=O stre.), 1668.29 (C=N stre.), 1591.23 & 1466.55 (Ar C=C stre.), 1233.67 (C-O-C stre.), 1189.48 (C-N stre.). ^{1}H NMR (DMSO, δ ppm) 8.314 (s, 2H, C(O)NH), 7.721 (s, 2H, NH_2), 6.765-7.539 (m, 4H, Ar-H),

6.445 (s, 1H, C=NH), 4.521-4.567 (m, 1H, OCH), 4.231(s, 3H, OCH_3), 3.715-3.722 (d, 2H, OCH_2), 3.379-3.395 (t, 2H, CH_2O), 3.222 (s, 1H, NH), 2.981-3.098 (t, 2H, NCH_2), 2.754-2.868 (m, 1H, NCH), 2.542 (s, 6H, $N(CH_3)_2$), 2.263-2.357 (t, 2H, C-CH_2-C), 1.754-1.868 (m, 2H, $CH_2CH_2CH_3$), 1.365-1.455(m, 2H, $CH_2CH_2CH_3$) 1.542-1.647 (t, 3H, CH_2CH_3),1.162-1.175 (d, 6H, $(CH_3)_2$). m/z 507 (M^+).

Chemical hydrolysis studies

Hydrolytic behavior of synthesized co-drugs was studied in Simulated Gastric Fluid (pH 1.2; USP 1970); Simulated Intestinal Fluid (pH 6.8); Simulated Plasma Fluid (pH 7.4; USP 1970). [21-23] The hydrolysis was performed by using USP-II paddle apparatus at a rotational speed of 50 rpm. 900 ml solution of pH 1.2, 6.8 and 7.4 were used as dissolution media and maintained at 37 ± 1°C. 1 ml of the hydrolysis medium was taken out at zero minute and every 15 min. for 120 min. 1 ml of the pH solution was added to the dissolution vessel. The sample withdrawn was subjected for HPLC analysis using Phenomenex Luna C_{18} column (250 mm × 4.6 mm id, 5µm 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 222 nm. The comparative study of rate of hydrolysis is shown as follows.

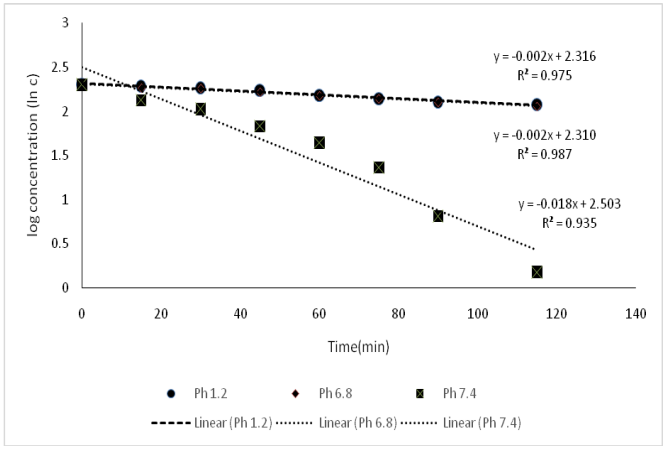


Fig. 2: The hydrolysis rate of compound COa at different pH values.

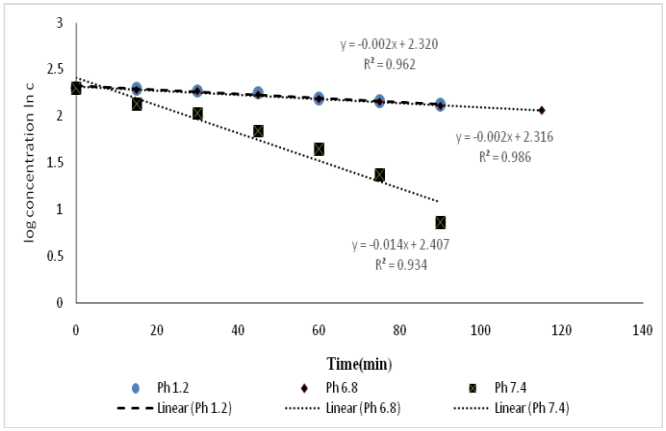


Fig. 3: The hydrolysis rate of compound COb at different pH values.

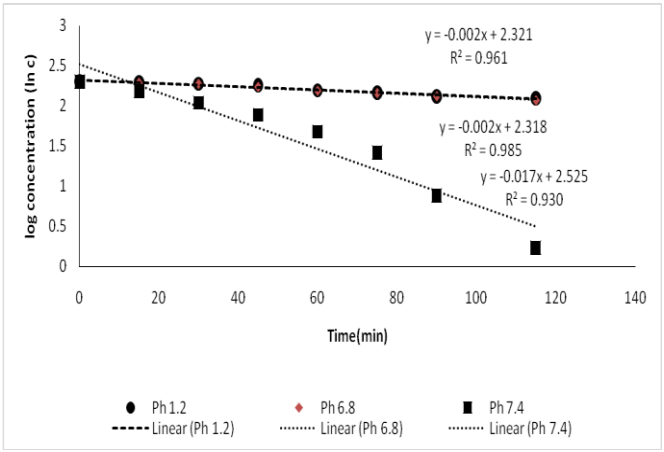


Fig. 4: The hydrolysis rate of compound COc at different pH values.

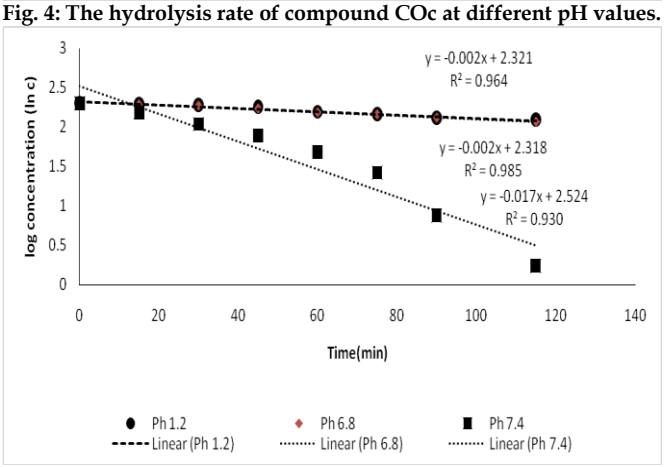


Fig. 5: The hydrolysis rate of compound COd at different pH values.

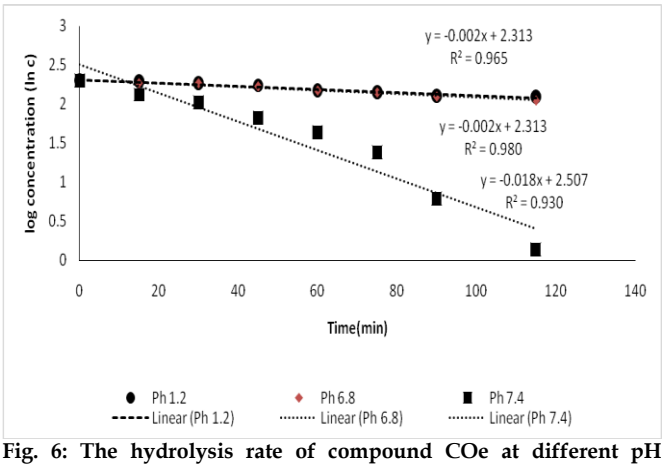


Fig. 6: The hydrolysis rate of compound COe at different pH values.

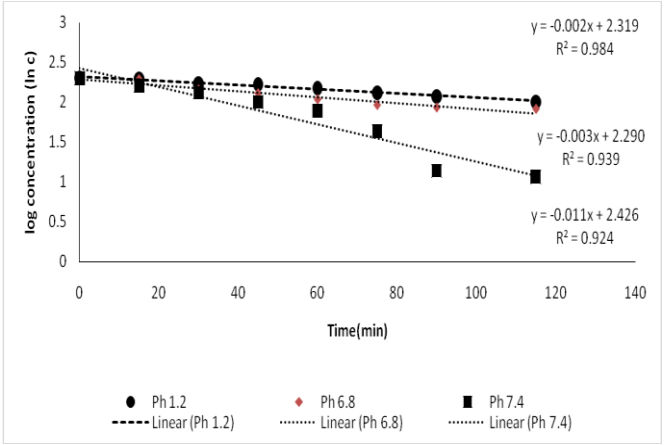


Fig. 7: The hydrolysis rate of compound COf at different pH values.

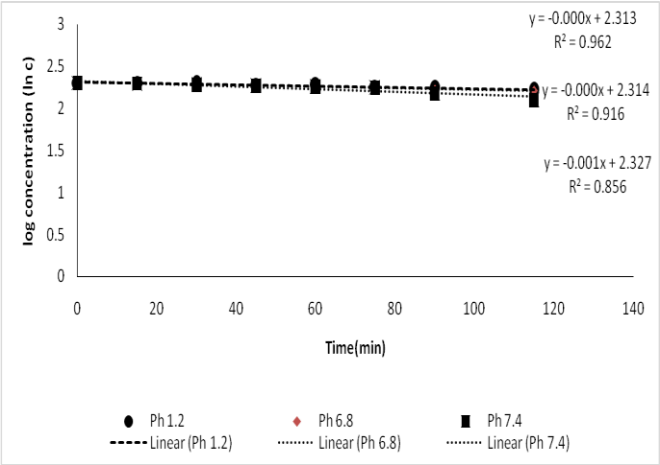


Fig. 8: The hydrolysis rate of compound COg at different pH values.

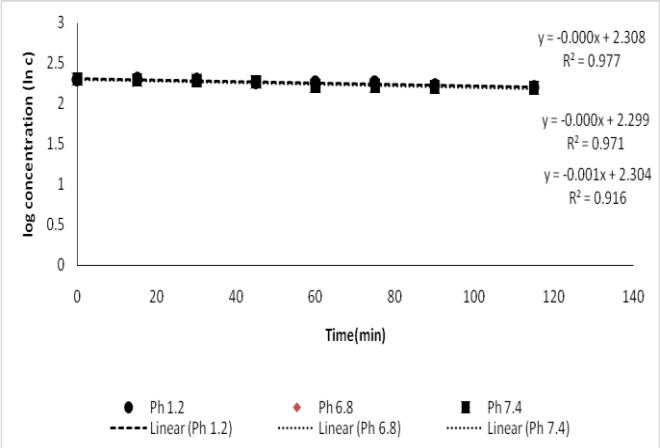


Fig. 9: The hydrolysis rate of compound COh at different pH values.

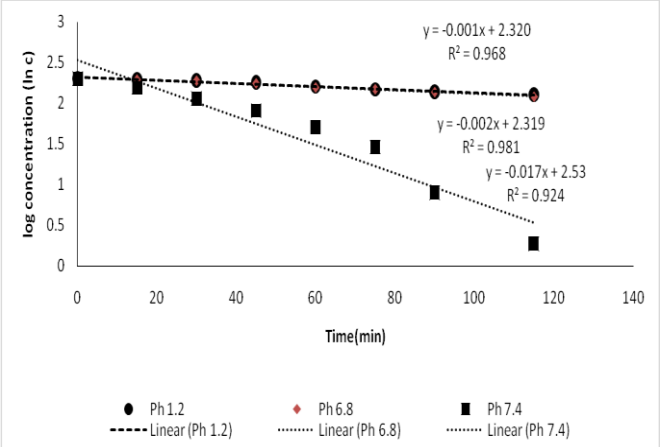


Fig. 10: The hydrolysis rate of compound COi at different pH values.

RESULTS AND DISCUSSION

Codrugs of metoprolol and metformin were prepared with an intend to enhance the bioavailability of drug in a time controlled drug delivery and thereby increasing the duration of action. The schematic representation of synthesis is mentioned in Figure 1. In the present work phthalic anhydride, various succinic and maleic anhydride derivatives were used to prepare the prodrugs Pa-i. The physicochemical characterization like melting point and spectral characterization by IR, 1H-NMR and mass spectral data were carried out for the synthesized prodrugs and 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 Rf value ensured completion of the reaction. The TLC plates were visualized either by iodine vapors or by viewing in UV-visible chamber. The reaction products of all the reactions were purified initially by different workup processes to remove unreacted starting materials if any and then by recrystallization using suitable solvents. The FTIR spectra of prodrug and co-drug showed the expected bands for the characteristic groups which are present in the compounds. The formations of a various prodrugs Pa-i were confirmed by the disappearance of IR band at 2840.70 - 3210.88 cm^{-1} for hydroxyl [–OH] group of carboxylic acid in the IR spectra's of all prodrugs and in co-drug's it is found to a new peak of amide carbonyl group [–NH(C=O)] in the range of 1732.69-1690.22 cm^{-1}.

In the ^1H NMR spectra, all protons were seen according to the expected integral values. The aromatic protons appeared in the range of 6.614 - 8.269 ppm. The ^1H NMR spectrum also supports the scheme of synthesis by the absence of peak appeared at δ value 12.832 – 13.849 ppm which corresponds to –COOH functional group indicating that the –COOH group was involved in the reaction. It was converted to amide by the formation of new peak at 8.144 - 8.664 ppm which confirms the formation of co-drugs COa-i of prodrugs Pa-i and metformin. The mass spectra of compounds gave molecular ions of medium intensity and the base peak usually belonged to the corresponding ions.

The kinetic of hydrolysis of the synthesized codrugs COa-i were studied in aqueous buffer solutions of pH 1.2 (non enzymatic Simulated Gastric Fluid, SGF), pH 6.8 (non enzymatic Simulated Intestinal Fluid) and pH 7.4 (Simulated Plasma Fluid) at 37±5°C using HPLC. The disappearance of the tested compounds displayed hydrolysis kinetics over the investigated pH and temperature. As a general pattern, the synthesized codrugs showed relative stability in the investigated aqueous solutions and the degradation rates at pH 7.4 are slightly accelerated than those observed in SGF of pH 1.2 and SIF of pH 6.8 except COg and h. COa-f and COi showed the more hydrolysis at pH 7.4 compared to pH 1.2 and 6.8, it indicate that the compounds containing two to four carbon atom chain irrespective with the saturation or unsaturation give the maximum hydrolysis.

The synthesis of codrugs of Metoprolol and 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, 1H NMR, and MS analysis. Absorption bands obtained in IR and NMR spectrum confirmed the formation of amide linkage between Metoprolol prodrugs and metformin. Preliminary kinetic study for compounds COa-i revealed that compounds were chemically stable to a great extent at

pH 1.2 and pH 6.8. While they shows a fast chemical hydrolysis at pH 7.4, with more hydrolysis for codrugs COa-f, COi and the COg and COh compounds were more stable relatively. It implies that codrugs may pass unhydrolyzed through stomach and posses enough stability to be absorbed from intestine. pH specific hydrolysis and slower hydrolysis of certain codrugs indicates the rate-controlled and time controlled drug delivery of the actives. It also translates in an increase in the duration of action, increase in the efficacy of drug. As the codrugs were stable at pH 1.2 and 6.8 this indicates towards avoiding the first pass metabolism and polypharmacy. 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 the active drugs for longer period of time. On the basis of chemical hydrolysis studies in this work, presence of maleate, methyl maleate, dimethyl maleate, ethyl maleate, propyl maleate, phenyl maleate, succinate and phthalate group may not appears to be suitable linker whereas methyl succinate appears to be a good linker.

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