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RESEARCH ARTICLE

Synthesis and Physicochemical Characterization of Metoprolol Prodrugs

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ABSTRACT

Metoprolol is widely used in the treatment of angina and hypertension. It shows great potential to treat sympathetic nervous system disorders. The success of metoprolol is limited due to its high first pass metabolism. Prodrug is one of the strategies to reduce the required dose of the drugs in order to achieve the desired bioavailability with reduced first pass metabolism. In the present study, three different prodrugs of metoprolol (metoprolol acetyl ester (MA-1), metoprolol acetamide (MA-2) and metoprolol benzamide (MA-3)) were synthesized. Further they were evaluated for physicochemical properties including solubility and partition coefficient. Ester prodrugs were found to be more soluble at pH 1.2 whereas amide prodrugs at pH 7.4 respectively showing the difference in solubility pattern. Both drug as well as prodrugs was found to be stable at pH 1.2 as compared to pH 7.4. Additionally introduction of ester and amide group in metoprolol increased the lipophillicity as observed in partition coefficient study. Prodrugs were found to be more lipophillic than metoprolol succinate. Both ester as well as amide prodrugs were found to be interesting for further in-vivo animal study.

KEYWORDS

Prodrug, Metoprolol acetamide, Metoprolol benzamide, Physicochemical parameters, Structure elucidation

INTRODUCTION

The ultimate goal of research in drug delivery is to develop strategies for delivering pharmacologically active agents to specific target sites in a highly efficient and controlled manner. In contrast to this ideal, present-day drug therapy is a highly challenging process due to a multitude of competing processes and biological barriers which combine to reduce the quantity of an administered drug reaching its intended target. An understanding is required to overcome these Controlled release system differs from other systems which simply prolong the drug release and hence the plasma drug levels for an extended barriers in order to achieve efficient drug delivery.

For orally administered drugs, first-pass liver uptake and metabolism is a formidable barrier to efficient delivery. Extensive first-pass metabolism, in addition to decreasing the percentage of dose reaching its intended site of action, often leads to serious variability in bioavailability. While first-pass metabolism can be avoided by selecting alternative routes of administration (transdermal, rectal, etc.), still oral administration is generally the preferred route of

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administration due to patient compliance. It would of great importance to design an alternative approaches for by passing first-pass metabolism which could be applied to drug candidates which exhibit promising pharmacological activity but undergo extensive first pass metabolism when administered orally.

There are a number of examples of the use of the prodrug approach to reduce first pass metabolism and thereby increase oral bioavailability[1]. For example, Dobrinska et a1. (2, 3) demonstrated that the highly lipophilic pivaloyloxyethyl ester of methyldopa exhibited a 2.3-fold higher systemic availability of methyldopa following an ora1 dose compared to an equivalent oral dose of methyldopa due at least partially to decreased first pass metabolism. Formation of the mono-O-sulfate conjugate of methyldopa was shown to be lower for the prodrug than for methyldopa[2,3]. Bodor et al., studying various classes of transient derivatives of L-dopa, which is used in the treatment of Parkinsonism[4], showed that derivatives such as the diacetyl-L-dopa ester, the benzyl ester and various dipeptides effectively provided protection against metabolism which occurred in the gastrointestinal tract and/or during the first passage through the liver, resulting in a significantly better bioavailability of the drug.

Metoprolol Succinate (MS), a β selective adrenergic blocking agent is a well-established drug in the treatment of angina pectoris, myocardial infarction and congestive heart failure as well as hypertension. Metoprolol reduces blood pressure by reduction 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. The transdermal route by virtue of its capability to avoid the hepatic first pass effect is projected to achieve higher systemic bioavailability. Hence this moderately high molecular weight (535 Da. pKa 9.5) drug has been investigated for skin permeability both by passive diffusion and iontophoretic technique[5-10]. So in the present study an attempt has been made to prepare different prodrugs of metoprolol and characterize them substantially including physicochemical parameters and their structure elucidation.

MATERIAL AND METHODS

Materials

Metoprolol succinate was obtained from Amneal Pharmaceuticals, Ahmedabad. Cesium carbonate and 1-octanol was purchased from Sigma Aldrich. All other solvents used were of analytical grade obtained from finar chemicals.

Methods

Synthesis of Prodrugs

Synthesis of Metoprolol Acetate (MA-1)

Metoprolol succinate was dissolved in water and was stirred till no particulate was observed. To the above solution saturated sodium carbonate solution was added and was stirred till effervescence sized out. The reaction mixture was extracted with dichloromethane (DCM) which upon vacuum distillation gave metoprolol base. Metoprolol base was dissolved in dichloromethane and to it IPA-HCl (hydrochloric acid dissolved in isopropyl alcohol) was added drop by drop under chilling condition at temperature between 2-5^{o}C. The DCM was distilled off under vacuum to give thick recipe which was triturated with hexane to give white crystalline material of Metoprolol Hydrocloride.

Metoprolol hydrochloride obtained above (0.9 gram, 3 mmol) was dissolved in dichloromethane (10 ml) at room temperature. Above solution was stirred at 2-5^{o}C for 15 minutes followed by addition of acetyl chloride (0.32 ml, 1.5 meq). After completion of addition the reaction mixture was then refluxed at 40^{o}C for overnight and the reaction completion was monitored on TLC (DCM: methanol 9:1 v/v).

The reaction mixture was cooled to room temperature and then the dichloromethane was distilled off under vacuum to give thick sticky material. This sticky material was triturated with hexane to yield white precipitates of Metoprolol acetate hydrochloride salt.

Metoprolol acetate hydrochloride salt was dissolved in water and was stirred till no particulate was observed. To the above solution saturated sodium carbonate solution was added and was stirred till effervescence sized out. The reaction mixture was extracted with dichloromethane (DCM) which upon vacuum distillation gave oily metoprolol acetate (MA-1, Yield 72%).

Synthesis of Metoprolol acetamide (MA-2)

Metoprolol (0.8 grams, 3 mmol) and triethyl amine (0.63 ml, 3 meq) were dissolved in toluene (10 ml) at room temperature. Above solution was stirred at 2-5ºC for 15 minutes followed by addition of acetyl chloride (0.32 ml, 3 meq). After completion of addition of acetyl chloride the reaction mixture was stirred at ambient temperature for and the reaction completion was monitored on TLC (DCM: methanol 9:1 v/v). After completion, the organic phase was treated twice with 0.1N hydrochloride acid and separated which was distilled off under vacuum to give oily metoprolol acetamide (MA-2, Yield 79%).

Synthesise of Metoprolol benzamide (MA-3)

Metoprolol (0.8 grams, 3 mmol) and triethyl amine (0.62 ml, 3 meq) were dissolved in toluene (10 ml) at room temperature. Above solution was stirred at 2-5ºC for 15 minutes followed by addition of benzoyl chloride (0.57 ml, 3 meq). After completion of addition of benzoyl chloride the reaction mixture was stirred at ambient temperature for and the reaction completion was monitored on TLC (DCM: methanol 9:1 v/v). After completion, the organic phase was treated twice with 0.1N hydrochloride acid and separated which was distilled off under vacuum to give oily metoprolol benzamide (MA-3, Yield 86%).

The synthesis of metoprolol prodrugs is outlined in following Figure 1.

Analysis of Prodrugs

Waters Alliance system with UV detector and analytical column Kromasil ^{100} RP-18(250 x4.6mm, 5µm) from Merck was used for metoprolol succinate and its pro-drugs' quantification. Metoprolol succinate and its pro-drugs' were separated and eluted isocratically at a flow rate of 1 ml/min using mobile phase concentration of Acetonitrile: Methanol: 10 mM aqueous Phosphate buffer (20:20:60). Injection volume was 20 µl and retention time of metoprolol was 3.19 min. Pro-drugs MA-1, MA-2 and MA-3 were eluted at 6.22, 5.79 and 7.42 minute respectively. Peaks were measured at a wavelength of 225 nm[11].

Physicochemical Properties[12,13]

Comparative study of metoprolol succinate and its prodrugs were performed which includes solubility study, solution state stability study and partition coefficients. The study was carried out to compare the physicochemical behavioral of metoprolol succinate and its prodrugs.

Solubility Measurements

A solubility study of MS, MA-1, MA-2 and MA-

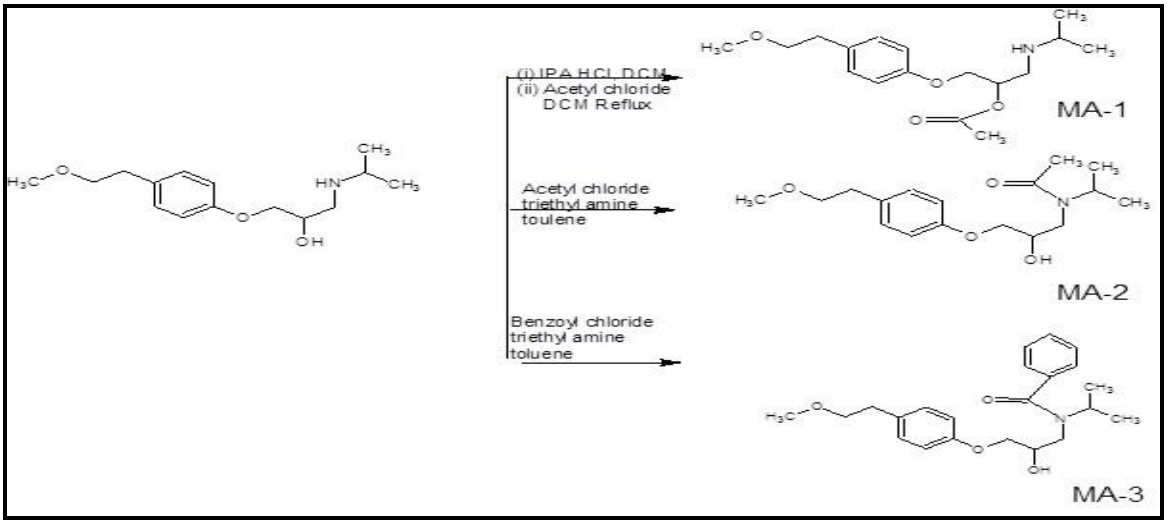

Figure 1: Synthesis pathway of metoprolol prodrugs

3 were carried out by the method of Okumara et al[14]. An excess amount of drug and prodrugs were added and dissolved in 10 ml of buffer (pH = 1.2, 4.5, 6.8 and 7.4) in a glass vial to get a saturated solution. The system was stirred for 24 h at 37˚C and kept at rest for 1 h to assist the attainment of equilibrium and centrifuged at about 5000 rpm for 30 minutes and isolated the supernatant. After dilution[15], the solubility of each drug and prodrugs were determined by HPLC.

Stability Study Metoprolol

The hydrolysis of the synthesized ester prodrugs was studied in 0.1 hydrochloride acid buffer (pH 1.2) and phosphate buffers (pH 7.4). The stock solutions (1 mg/ml) of MS, MA-1, MA-2 and MA-3 were prepared and dissolved in 10 ml of buffer solutions of different pH 1.2 and 7.4. A constant ionic strength (μ) of 0.5 was maintained for both buffers by adding a calculated amount of potassium chloride.

The rates of hydrolysis were determined by using HPLC method. Quantification of the ester as well as free drug formed upon hydrolysis was done by measuring the peak height in relation to those of standard chromatogram under the same condition. The hydrolysis studies were carried out for 24 hours[16].

Measurement of Partition Coefficient

The partition coefficient of drug and prodrugs were determined in mutually saturated (at 250˚C for 24 h) n-octanol–pH 7.4 buffer (μ = 0.5) system. The partition coefficient study was performed using mixture of 450 µl of n-octanol and 450 µl of phosphate buffer pH 7.4. This mixture of was shaken in thermomixer at 37º C for 24 hours to achieve equilibrium. The 100 µl of drug and prodrug solution (1 mg/ml in DMSO) was added then again it was shaken for 4 hours in thermomixer and centrifuged to separate the layers. Both the layers were injected to HPLC and drug content was calculated. The partition coefficient was determined from the equation:

Partition coefficient = Conc.in octanol layer/Conc.in buffer layer

Structural Characterization

The synthesized metoprolol prodrugs were characterized by FTIR, ^{1}H-NMR and Mass to confirm its structures.

FTIR Spectroscopy

The FTIR spectra of MS and prodrugs were recorded by FTIR instrument (Perkin Elmer G-FTIR, Waltham, MA). Samples in the dried form were crushed and mixed with KBr in the ratio of approximately 1:3. The IR spectra were done against the KBr background. Spectral scanning was done in the range between 4000 and 400cm^{-1}.[17]

NMR Spectroscopy

The binding of coating to the core in iron–sucrose formulations could be evaluated by proton NMR spectroscopy. The samples were dissolved in $CDCl_3$ to make 5% solutions. The 5% sample solutions were transferred to 5 mm NMR tube and the spectra were recorded using Bruker Advance II (400 MHz) NMR Spectrometer (Bruker BioSpin AG, Fllanden, Switzerland)[18].

Mass Spectroscopy

Mass spectrometry measures the mass of molecules by measuring the mass-to-charge ratio (m/z). Molecular mass analysis was performed by Electron impact (EI) ionization technique (Empower Pro)[18].

RESULTS AND DISCUSSION

Analysis of Prodrugs

The quantification of metoprolol prodrugs by RP HPLC method significantly separated the peaks of MS and three prodrugs. This infers that synthesized prodrugs are different. Table 1 indicates the retention time of different prodrugs.

Table 1: Retention time of MS and metoprolol prodrugs

Prodrug	Retention time
MS	3.19 min
MA-1	6.22 min
MA-2	5.79 min
MA-3	7.42min

Physicochemical Properties

Solubility Study

Solubility of Metoprolol and its prodrugs (MA-1), (MA-2) and (MA-3) was performed in different buffers. Table 2 shows that MS is comparably soluble in pH 4.5 buffer as compared to highly acidic and neutral pH.

Whereas, prodrug (MA-1) is more soluble in acidic pH as compared to neutral pH. This may be due to the presence of ionisable amino group in the prodrug. MA-2 and MA-3 have more solubility at pH 7.4 as compared to pH 1.2.

Table 2: Solubility of MS and prodrugs in various buffers (Mean ± S.D.)

Drug	Solubility (mg/ml) at given pH			
	1.2	**4.5**	**6.8**	**7.4**
MS	0.59 ± 0.13	1.35 ± 0.16	ND	ND
MA-1	ND	2.06 ± 0.14	0.39 ± 0.06	0.45 ± 0.06
MA-2	0.85 ± 0.08	0.62 ± 0.07	1.34 ± 0.1	1.60 ± 0.19
MA-3	0.68 ± 0.05	0.84 ± 0.07	1.62 ± 0.28	1.53 ± 0.16

(ND= Not Determined)

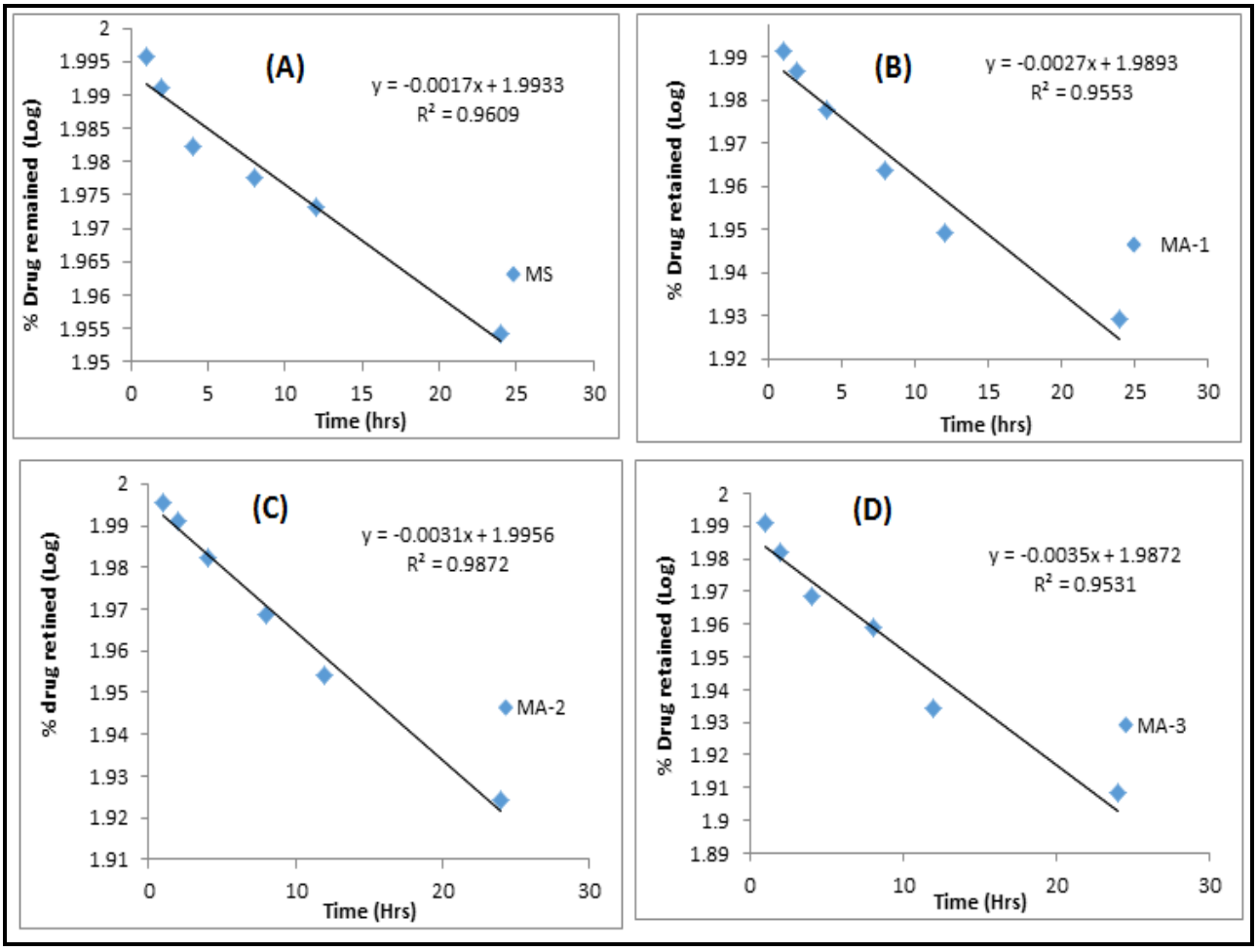

Figure 2: Solution state stability at pH 1.2 of (A) MS (B) MA-1 (C) MA-2 (D) MA-3

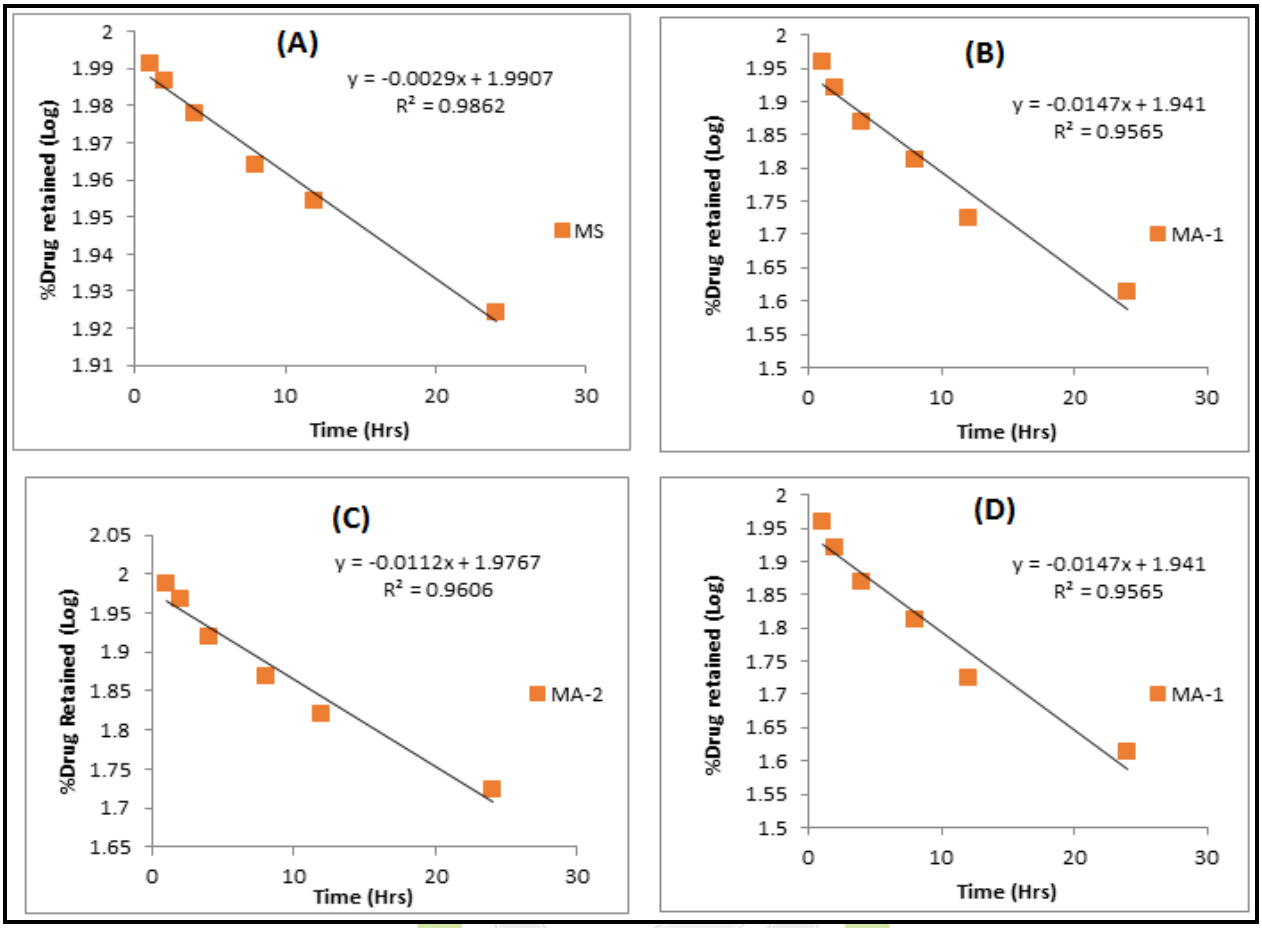

Figure 3: Solution state stability at pH 7.4 of (A) MS (B) MA-1 (C) MA-2 (D) MA-3

Stability Study Metoprolol Prodrugs

The stability of the prodrug in different pH 1.2 and pH 7.4 is carried out to determine the stability of drug or prodrug before absorption.

Figure 2 and Figure 3 indicates that the drugs and prodrugs are more stable in the acidic media than basic media. All prodrugs were found to be stable enough so that they can be absorbed in their intact form before getting degraded. Moreover the data indicates that ester or amide linkage is more prone to cleavage under basic condition than acidic media. All prodrugs are found to be quite stable at pH 1.2 where it shows decrease in concentration at pH 7.4 in longer duration. Additionally degradation of MS, MA-1, MA-2 and MA-3 follows first order kinetics at pH 1.2 and pH 7.4.

Determination of Partition Coefficient of Metoprolol Prodrugs

Partition coefficient study mainly highlights the change in the order of lipophilicity. Table 3 shows the Log P values of metoprolol and its prodrugs which clearly indicate that all metoprolol prodrugs are more lipophillic than MS. Thus it infers that prodrugs have enhanced lipophilicity and consequently permeability.

Table 3: Partition coefficient of Metoprolol prodrugs

Drug/ Prodrug	Log P at buffer pH 7.4
MS	-0.72 ± 0.02
MA-1	0.53 ± 0.04
MA-2	0.41 ± 0.02
MA-3	0.54 ± 0.02

(Mean± S.D.)

Structural Characterization

FTIR Spectroscopy

The characteristics peaks of the MA-1 were 3464 cm^{-1} and 1744 cm^{-1} which are due to -NH stretch and -COOR stretch group respectively. The characteristics peaks of the MA-2 were 3456 cm^{-1} and 1643 cm^{-1} which are due to -OH broad and -CONH- stretch groups respectively. The characteristics peaks of the MA-3 were 3336 cm^{-1} and 1671 cm^{-1} which are due to -OH broad and -CONH- stretch groups respectively. The respective FTIR spectra of MA-1, MA-2 and MA-3 are presented in Figure 4, 5 and 6 respectively.

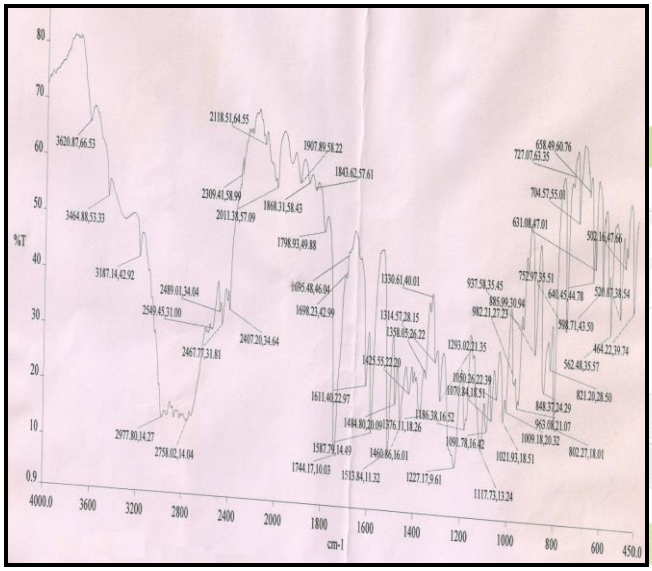

Figure 4: FTIR spectrum of MA-1

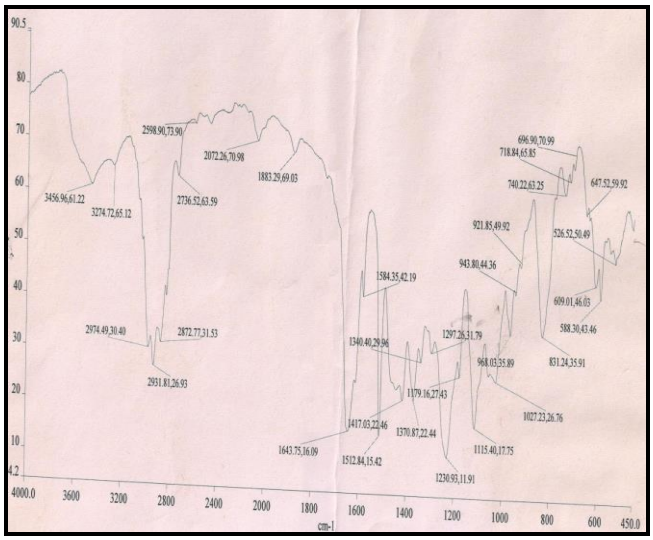

Figure 5: FTIR spectrum of MA-2

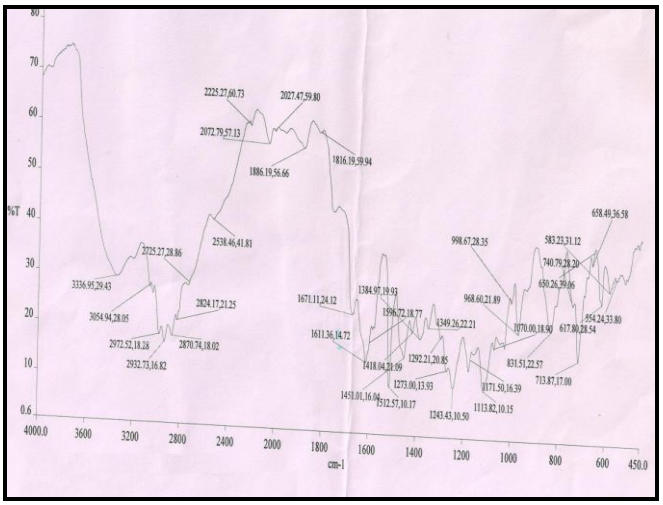

Figure 6: FTIR spectrum of MA-3

NMR Spectroscopy

MA-1 ^{1}H NMR ($CDCl_3$)

1.16-1.40 (q, 6H (-NCH(CH_3)));2.22 (s, 3H (-$COCH_3$)); 2.80-2.82 (t, 2H (CH_2)); 3.34 (s, 5H (-CH, NCH_2, -OCH_2); 3.53-3..57 (t, 3H(-OCH_3)); 4.18-4.20 (m, 2H(-CH_2)), 5.58-5.68 (m, 1H (-OCH-)); 6.81-6.84 (dd, 2H(aromatic-CH));7.11-7.26 (dd, 2H(aromatic-CH)); 9.29 (bs, 1H(-NH)

MA-2 ^{1}H NMR ($CDCl_3$)

1.19-1.28 (m, 6H (-NCH(CH_3))); 2.04 (s, 3H(-$NCOCH_3$)); 2.13 (s, 2H (CH_2)); 2.17-2.19 (d, 1H(-OH)); 2.80-2.84 (m,2H(-OCH_2)); 3.34 (s,3H (-OCH_3)); 3.53-3.59 (m, 2H (-NCH_2)); 3.66-3.73 (m, 1H(-NCH)); 3.99-4.01 (m, 2H(-OCH_2)); 4.08-4.13 (m, 1H(-CH-)); 5.36- 5.40 (bs, 1H(-OH)); 6.81-6.85 (dd, 2H(aromatic-CH)); 7.10-7.16 (dd, 2H(aromatic-CH));

MA-3 ^{1}H NMR ($CDCl_3$)

1.11-1.24 (m, 6H (-NCH(CH_3))); 2.30-2.31 (d, 2H (-CH_2)); 2.79-2.85 (m, 2H(-CH_2); 3.35 (s, 3H (-OCH_3)); 3.47-3.58 (m, 2H (-OCH_2)); 3.73-3.78 (m; 1H (-NCH)); 3.87-3.92 (t, 2H (-OCH_2)); 3.97-4.11 (m, 1H (-CH)); 6.83-6.88 (m, 2H (aromatic-CH)); 7.06-7.21 (m, 2H (aromatic-CH)); 7.28-7.58 (m, 3H (-NHCO-aromatic-CH)); 8.02- 8.08 (m, 1H (-NH))

Mass Spectroscopy

The characteristics peak of MA-1 and MA-2 in mass spectroscopy were observed at m/z 310 [M+1] whereas MA-3 give its characteristic peak

at m/z 371 [M+1] as shown in Figure 7, 8 and 9 respectively for MA-1, MA-2 and MA-3.

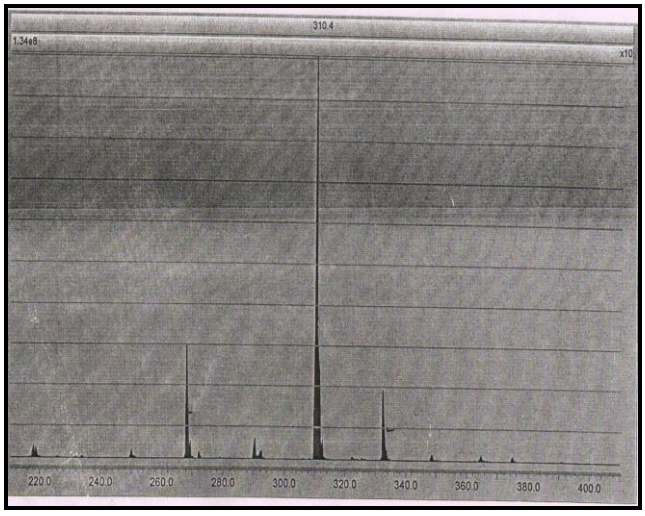

Figure 7: Mass spectrum of MA-1

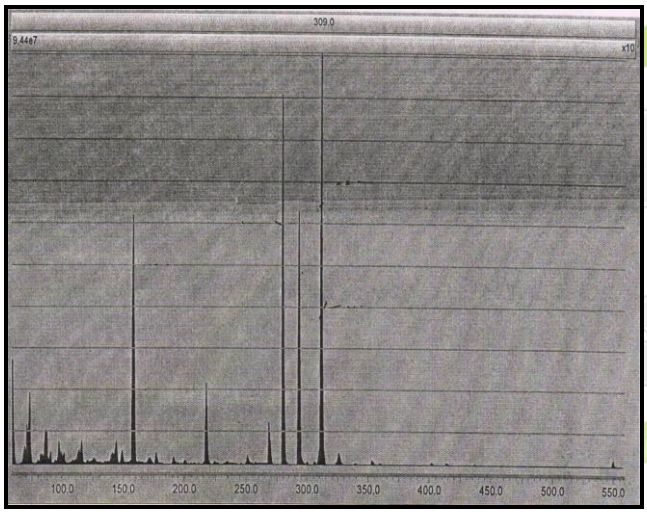

Figure 8: Mass spectrum of MA-2

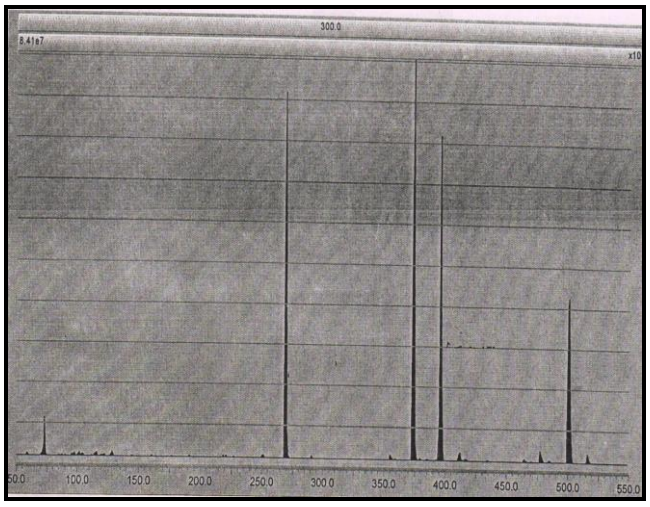

Figure 9: Mass spectrum of MA-3

CONCLUSION

Introduction of ester or amide group as a prodrug strategy greatly affects the physicochemical behavior as seen from the data obtained. The solubility of ester prodrug MA-1 is more in acidic media whereas amide prodrugs MA-2 and MA-3 are slightly more soluble at neutral pH. Additionally, esters are more prone to cleavage under basic condition as compared to amide prodrugs indicating that amide prodrugs are more stable. Additionally the difference between drug and prodrugs were observed in order of lipophillicity. Introduction of lipophillic group i.e. ester or amide into metoprolol enhances the lipophillicity which may warrant the enhancement in permeability across cell membrane. Further in-vivo study of the MS and prodrugs will be carried out to compare the difference in bioavailability of metoprolol from MS and prodrugs respectively.

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