CHAPTER - 2

SYNTHESIS AND CHARACTERIZATION

OF

NOVEL ANTI-LIPIDEMIC AGENTS
2.1 - INTRODUCTION

2.1.1 - Drug Discovery and Anti-lipidemic agents:

Anti-lipidemic agents are basic drugs for prevention of cardiovascular diseases and have been in use for more than 4 decades. Anti-Lipidemic agents are categorized into various types like (i) Niacin, (ii) Fibrates (fenofibrate [Tricor®], gemfibrozil [Lopid®], clofibrate [Atromid-S®], bezafibrate), (iii) Resins that bind bile Acid (colestipol [Colestid®] and cholestyramine [Questran®]), and (iv) HMG COA Reductase Inhibitors (“statins”). The first class of drugs with an established efficacy in lipid-lowering phenomenon in humans were 3-hydroxy-3-methyl glutaryl coenzyme A (HMG COA) reductase inhibitors (statins). 1

HMG-CoA reductase inhibiting drug candidates, which are called as HMG-CoA reductase inhibitors (or "statins") are used to reduce cholesterol in serum as a method of risk reduction for the cardiovascular disease.2

2.2 –LITERATURE REVIEW

In view of the importance of hydrophilicity of statins discussed in Introduction Chapter-1, it is hypothesized that introduction of more hydrophilic scaffolds into the statin compounds by linking the pharmacophore ‘dihydroxy heptanoic acid moiety’ with more hydrophilic scaffolds to result in compounds, which may act as better anti-lipidemic agents especially as HMG-CoA reductase inhibitors, expected to improve
the level of hepato selectivity and expected to reduce the risk of adverse effects.

Pfizer’s Lipitor was the world’s top-selling medicine during the year 2010, according to IMS (International Market Survey) database, ranking in sales of $13.3billion. AstraZeneca’s Crestor, which garnered $5.38billion in sales during 2009, is one of the company’s best-selling medicines.

US patents of all the major selling statins are expected to expire by 2012. Lovastatin (patent expiry-June, 2001), Provastatin (patent expiry-April, 2006), Simvastatin (patent expiry-June, 2001), Atorvastatin (patent expiry-June, 2011), Fluvastatin (patent expiry-April, 2012) & Rosuvastatin (patent expiry-June, 2012).

2.3 – PRESENT WORK

In view of the great market potential and unmatched therapeutic efficacy of statins, new possibilities have been opened up for the rational design and optimization of even better HGMR inhibitors. In the present work, we have invented new HMG-CoA reductase inhibitors, which are easier to synthesize and ensured that all the newly invented inhibitors are retained with key pharmacophore ‘dihydroxy heptanoic acid moiety’.
2.4 - RESULTS AND DISCUSSION - SYNTHESIS OF NOVEL ANTI-LIPIDEMIC AGENTS:

The starting point for the synthesis of novel anti-lipidemic agents of the present work is tert-butyl 2-[(4R,6R)-6-(2-aminoethyl)-2,2-dimethyl-1,3-dioxan-4-yl]acetate (1), a key intermediate of the statin drug Atorvastatin, which contains the derivatized ‘dihydroxy heptanoic acid moiety’. (1) was prepared according to the synthetic process described for the same in was prepared from Ethyl (R)-4-Cyano-3- hydroxy butyrate by a procedure similar to that described in the US patent Butler et al. 23

To this, relatively polar scaffolds were embedded when compared to most of the existing statins in order to act as more hydrophilic groups towards the objective of the present work. The hydrophilic building block was introduced by adapting the following synthetic approach (scheme-2.1).

(i) reacting epichlorohydrin with (1) by refluxing in IPA to afford tert-butyl 2-[(4R,6R)-6-(2-((3-chloro-2-hydroxypropyl)amino)ethyl)-2,2-dimethyl-1,3-dioxan-4-yl]acetate (2);

(ii) (2) was converted to isopropyl 2-[(4R,6R)-6-(2-((1,3-dioxoisoindolin-2-yl)methyl)-2-oxooxazolidin-3-yl)ethyl]-2,2-dimethyl-1,3-dioxan-4-yl]acetate (3). The said conversion was experimented in two approaches as follows:
(ii)-(a) (2) was reacted with carbonyldiimidazole in presence of 4-DMAP in THF as reaction medium at room temperature to build the heterocycle oxazolidinone and resulted in tert-butyl 2-((4R,6R)-6-(2-(5-(chloromethyl)-2-oxooxazolidin-3-yl)ethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (4) followed by reacting it with potassium phthalimide in TEA at 70°C afford (3); or

(ii)-(b) (2) was first reacted with potassium phthalimide in TEA at 70°C to afford tert-butyl 2-((4R,6R)-6-(2-(3-(1,3-dioxoisoindolin-2-yl)-2-hydroxypropyl) amino)ethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (5) followed by reacting it with CDI in presence of 4-DMAP in THF as reaction medium at room temperature to build the heterocycle oxazolidinone to afford (3);

(iii) hydrolysis of (3) using aq. MeNH₂ in MeOH under refluxing resulted in tert-butyl 2-((4R,6R)-6-(2-(5-(aminomethyl)-2-oxooxazolidin-3-yl)ethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (6);

(iv) derivatization of the amine (6) by acylation or alkylation using appropriate acylating agent or alkylating agent lead to various acyl amino or alkyl amino compounds of formula (7); wherein R is alkyl or acyl group;

(v) deprotection of acetonide moiety of (7) using aq. HCl in acetonitrile reaction medium at room temperature resulted in dihydroxy ester compounds of formula (8); wherein R is alkyl or acyl group;
(vi) hydrolysis of the resulting ester afforded the targeted statin compounds of formula (9); wherein R is alkyl or acyl group either in their free acid form or in the form of their base addition salts.

Conversion (ii)-(b) was favourable, whereas conversion (ii)-(a) was not favourable and therefore was not studied further.

**Scheme-2.1:**

(R = Isobutyryl, Acetyl, Benzoyl, Benzyl and 4-methyl benzyl)

(i) IPA, reflux; (ii) TEA, 70°C; (iii) CDI, 4-DMAP, THF, room temperature; (iv) Aq. MeNH₂, MeOH, reflux; (v) acyl anhydride or alkyl halide, pyridine, room temperature; (vi) aq. HCl, acetonitrile, room temperature; (vii) aq. NaOH, acetonitrile, room temperature, 45°C.
Various novel compounds prepared according scheme-2.1 of the present work and their structural characterization is discussed in more detail in the experimental section here under.

2.5 - EXPERIMENTAL SECTION

Preparation of (2):

A solution of (1) (1.0 g, 0.0036 mol) and epichlorohydrin (0.43 g, 0.0046 mol) in IPA and mixture was refluxed for 5 hours and evaporated under reduced pressure. The resultant (2) was purified by flash-LC (mixture of n-hexane and ethyl acetate in the 7:3 ratio) to give the title (2) (1.0 g, 78% yield) as a pale yellow syrup.

Characterization of (2):

IR spectrum of (2):

(cm\(^{-1}\)) 3350 (O-H str), 3200 (N-H str), 1750 (C=O str, ester).
Mass spectrum of (2) (Turbo spray):

m/z 366 (M+1).
Preparation of (5):

A mixture of (2) (0.5 g, 1.14 mmol) and potassium phthalimide (0.33 g, 1.78 mmol) were taken in TEA (2.5 mL). The reaction mixture was stirred for 4 hours at 70 °C. The reaction mixture was allowed to cool to room temperature and stirred for 1 hour before water (10 mL) was added. After addition of DCM and phase separation, the aqueous phase was extracted with DCM. The combined organic phases were dried (anhyd. sodium sulfate), filtered and evaporated under reduced pressure. The residue was purified by Flash-LC (mixtures of DCM/MeOH) to give the title (5) (0.46 g, 70% yield) as a colorless solid. mp: 230 °C.

Characterization of (5):

**IR spectrum of (5):**

(\(\text{cm}^{-1}\)) 3250 (O-H str); 3100 (N-H str); 1750 (C=O str, ester) and 1700 (C=O str, amide).
**Fig. 2.3**

*Mass spectrum of (5) [ESI]:*

m/z 478 (M+2).
Preparation of (3):

CDI (0.7 g, 4.31 mmol) and 4-DMAP (0.24 mmol) were added to a suspension of (5) (0.5 g, 1.68 mmol) in THF (8.4 mL). The reaction mixture was stirred for 3 h at room temperature and 3 h at 60 °C. The precipitate was filtered, washed with THF, and dried in vacuo. The combined mother liquors were evaporated and the resulting residue was purified by Flash-LC (mixtures of DCM/MeOH). The title (6) (0.46 g, 55% yield) was obtained as a colourless solid.

Characterization of (3):

Mass spectrum of (3) (ESI):

m/z 503 (M+1).

Fig. 2.5
$^1$H-NMR spectrum of (3) (CDCl$_3$, 400 MHz):

(δ ppm) 1.2 (s, 15H, 5XCH$_3$); 1.4 (m, 2H, CH$_2$ of acetonide); 1.5 (m, 2H, CH$_2$ adjacent to acetonide); 1.7 (m, 2H, CH$_2$ adjacent to ester); 2.2 (m, 2H, CH$_2$ adjacent to oxazolidinone); 2.9 (m, 2H, CH$_2$ of oxazolidinone); 3.1 (m, 2H, CH$_2$ attached to phthalimide); 3.6 (m, 1H, second CH of acetonide); 4.4 (m, 1H, one CH of acetonide); 5.0 (m, 1H, CH of oxazolidinone); 7.5-7.8 (m, 4H, Ar-H).

**Fig. 2.6**

**Preparation of (6):**

Methylamine (40% in water, 0.77 mL, 9.9 mol) was added to a suspension of (3) (0.5 g, 0.99 mmol) in MeOH (8 mL). The reaction
mixture was refluxed for 1 h and evaporated under reduced pressure. The resultant \((6)\) was purified by Flash-LC (mixtures of DCM/MeOH) to afford \((6)\) (0.70 g, 70% yield) as a colorless solid.

**Characterization of \((6)\):**

**IR spectrum of \((6)\):**

\((\text{cm}^{-1})\) 3400 (N-H str); 1700 (C=O str, ester), 1690 (C=O str, amide).

![Fig. 2.7](image)
**Mass spectrum of (6) (ESI):**

\[ m/z \ 373 \ (M^+ + 1). \]

![Fig. 2.8](image)

**\(^1\)H-NMR spectrum of (6) (CDCl\textsubscript{3}, 400 MHz):**

(\(\delta\) ppm) 1.3 (s, 6H, CH\textsubscript{3} of acetonide); 1.5 (s, 9H, CH\textsubscript{3} of ter-butyl); 1.6 (m, 2H, CH\textsubscript{2} adjacent to acetonide on right); 1.7 (m, 2H, CH\textsubscript{2} of acetonide); 2.0 (m, 2H of CH\textsubscript{2} between ester and acetonide); 2.1 (m, 2H, CH\textsubscript{2} adjacent to -NH\textsubscript{2}); 2.9 (m, 2H, CH\textsubscript{2} adjacent to N of oxazolidinone), 3.1 & 3.2 (m, 2H, CH\textsubscript{2} of oxazolidinone); 3.7 (m, 1H, CH of acetonide on oxazolidinone side); 4.5 (m, 1H, second CH of acetonide on ester side); 4.9 (m, 1H of CH of oxazolidinone); 5.4 (m, 2H, NH\textsubscript{2}).
Preparation of tert-butyl 2-((4R,6R)-6-(2-(5-(isobutyramidomethyl)-2-oxooxazolidin-3-yl)ethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (10):

Isobutyric anhydride (0.44 g, 2.8 mmol) was added drop-wise to a solution of the (6) in pyridine (8 mL) at 0°C. The reaction mixture was allowed to warm to room temperature and stirred for 1 h before water was added. After addition of DCM and phase separation, the aqueous phase was extracted with DCM. The combined organic phases were dried (sodium sulfate), filtered, and evaporated in vacuo. The residue was
purified by Flash-LC (mixtures of DCM/ MeOH) to afford (10) (0.41 g, 62% yield) as a colorless solid.

**Characterization of (10):**

**Mass spectrum of (10) (ESI):**

m/z 443 (M+1).

![Fig. 2.10](image)

**1H-NMR spectrum of (10) (CDCl₃, 400 MHz):**

(δ ppm) 1.3 (broad s, 12H, CH₃ of acetonide & CH₃ of isopropyl); 1.5 (s, 9H, CH₃ of ter-butyl); 1.6 (m, 2H, CH₂ adjacent to acetonide on right); 1.7 (m, 2H, CH₂ of acetonide); 2.0 (m, 2H of CH₂ between ester and acetonide); 2.1 (m, 2H, CH₂ adjacent to -NH); 2.6 (m, 1H, CH of isopropyl); 2.9 (m, 2H, CH₂ adjacent to N of oxazolidinone), 3.1 (m, 2H, CH₂ of oxazolidinone); 3.7 (m, 1H, of CH of acetonide on oxazolidinone side); 4.6 (m, 1H, second CH of acetonide on ester side); 4.7 (m, 1H, CH of oxazolidinone); 8.1 (m, 1H, NH).
By following the similar procedure involving the use of appropriate conventional reagents, four other derivatives (11-14) were synthesized.
Preparation of (3R,5R)-tert-butyl 3,5-dihydroxy-7-(5-(isobutyramidomethyl)-2-oxooxazolidin-3-yl)heptanoate (15):

A stirred mixture of (0.5 g, 1.1 m mol) of tert-butyl 2-((4R,6R)-6-(2-(5-(isobutyramidomethyl)-2-oxooxazolidin-3-yl)ethyl)-2,2-dimethyl-1,3-dioxan-4-yl)acetate (10) in 10 ml acetonitrile was added a diluted solution of 2.5 ml (2.2 mmol) of conc. HCl in 5 ml of water slowly at room temperature. Aged at room temperature for 45 minutes and pH of the reaction mixture was adjusted to 8 with aqueous sodium carbonate solution by controlling temperature at room temperature. The product was extracted with DCM and the organic layer was washed with water. Solvent was distilled under reduced pressure and the resulting (15) was purified by flash-LC (mixtures of DCM/MeOH) to (15) (0.2 g, 44% yield) as a colorless solid.

Characterization of (15):

**IR spectrum of (15):**

\( \text{cm}^{-1} \) 3350 (O-H str); 3200 (N-H str); 1690 (C=O str, ester); 1650 (C=O str, amide).
Mass spectrum of (15) (ESI):

m/z 403 (M⁺+1).
**1H-NMR spectrum of (15):**

(δ ppm) 1.3 (m, 15H, CH₃); 1.6-3.1 (m, 13H, CH₂ & CH of i-Pr); 3.5 (s, 2H, OH); 3.7 (m, 1H, second CH of CHOH); 4.5 (m, 1H, one CH of CHOH); 4.7 (m, 1H, CH oxazolidinone); 7.8 (m, 1H, NH).

By following the similar procedure involving the use of appropriate conventional reagents, four other derivatives (16-19) were synthesized.

![Images of compounds 16 to 19]

**Preparation of (3R,5R)-3,5-dihydroxy-7-(5-(isobutyramidomethyl)-2-oxooxazolidin-3-yl)heptanoic acid (20):**

![Image of compound 20]

A stirred mixture of (0.2 g, 0.5 mmol) of (3R,5R)-tert-butyl 3,5-dihydroxy-7-(5-(isobutyramidomethyl)-2-oxooxazolidin-3-yl)heptanoate (15) in 10 ml of acetonitrile was slowly added a solution of NaOH prepared by dissolving (25 mg, 0.6 mmol) of NaOH in 5 ml of water. The temperature was raised to 45°C and aged for 1 hour. Reaction mixture was cooled to room temperature and the pH was adjusted to 6 using
aqueous acetic acid. The product was extracted with DCM and the organic layer was washed with water. Solvent was distilled under reduced pressure and the resulting (20) was purified by flash-LC (mixtures of DCM/MeOH) to afford (20).

(20) was converted to calcium salt by first dissolving the acid in one equivalent of NaOH dissolved in water and then treating with 0.5 equivalent of calcium acetate to get the corresponding calcium salt.

**Characterization of (20):**

**IR spectrum of (20):**

(\text{cm}^{-1}) 3650 (N-H str); 3200 (O-H str, CHOH); 1700 (C=O str, acid); 1650 (C=O str, amide).

![Fig. 2.14](image)
**Mass spectrum of (20) (ESI):**

m/z 347 (M^+1).

![Mass spectrum of (20) (ESI)](image)

**1H-NMR spectrum of (20) (CDCl₃, 400 MHz):**

(δ ppm) 1.3 (m, 6H, CH₃); 1.4 (m, 2H, CH₂ between two CH-OH groups); 1.7 (2H, CH₂ of CH₂-CH₂-NH); 2.3 (m, 2H, CH₂ adjacent to COOH); 2.4 (m, 1H, CH of i-pr); 2.5 (m, 2H, CH₂ adjacent to N of oxazolidinone); 2.6 (m, 2H, CH₂ of oxazolidinone); 3.0 (m, 2H, CH₂ adjacent to NH); 3.3 (m, 1H, CH of one CH-OH); 3.5 (s, 2H, OH); 3.6 (m, 1H, CH of second CH-OH); 5.2 (m, 1H, CH of oxazolidinone); 7.5 (s, 1H, COOH); 7.7 (m, 1H, NH).
By following the similar procedure involving the use of appropriate conventional reagents, four other derivatives (21-24) were synthesized.
2.5 - CONCLUSION

The scaffolds containing oxazolidinone heterocycle, the amide and amino groups that are attached to the pharmacophore ‘dihydroxy heptanoic acid moiety’ of the statin compounds obtained in the present work are more polar when compared to most of the existing statins depicted in Fig. 1.1 and hence are considered to possess more hydrophilicity in order to give rise to the desired compounds that are useful as anti-lipidemic agents especially as HMG-CoA reductase inhibitors, which are expected to improve the level of hepatoc selectivity and reduce the risk of adverse effects such as diarrhoea, abdominal pain, constipation, flatulence etc.

The article related to novel anti-lipidemic agents and their process for preparation of the present work has been published as Chandra et al. (i)