DISCUSSION
Currently available antihyperlipaemic drugs decrease serum lipid levels by different mechanisms. Fibrates act by limiting substrate availability for triglyceride synthesis in the liver and promoting the action of lipoprotein lipase. They also increase the clearance of LDL from circulation by the LDL-receptor pathway (Rifkind, 1966; Nikkila et al., 1977; Shepherd, 1993). Probufol enhances the fractional catabolic rate of LDL by receptor-dependent as well as receptor-independent pathways (Yamamoto et al., 1983), and prevents oxidative modification of LDL (Steinberg & Witztum, 1991). Nicotinic acid and its derivative reduce the plasma cholesterol, which is due to decrease in production of VLDL. They also cause suppression of free fatty acid mobilisation from adipose tissue stores (Grundy et al., 1981). HMG-CoA inhibitors like lovastatin, dalvastatin, pravastatin, simvastatin etc., block the synthesis of cholesterol in the liver, thereby triggering compensatory reactions and finally reducing plasma LDL (Grundy & Bilheimer, 1984). Bile acid binding resins like cholestyramine and colestipol bind to the bile acids in the gut, interrupt their normal enterohepatic recirculation and increase faecal bile acid excretion (Moore et al., 1986). This increases the conversion of cholesterol into bile acids in the liver by the enzyme 7-alpha dehydrogenase (Grundy et al., 1971). The present investigation, provides at least four sets of evidences which suggest that the compound LM-1554 acts by inhibition of absorption of cholesterol from the gastrointestinal tract.

The first set of evidences comes from the results of the pharmacokinetic study of the compound LM-1554, which reveals that it is poorly absorbed from the gastrointestinal tract. Intravenous administration of 1 mg/kg in dogs produced 11.6 ± 0.93 μg/ml serum concentration of the compound after 10 mins. It was surprising that oral administration of 100 times higher dose (100 mg/kg) of LM-1554 in dogs produced the serum concentration of the compound 19.20 ± 1.70 μg/ml at tmax. Identical results were obtained in rabbits. The compound LM-1554 was isolated unchanged 45.8 % in faecal matter of rabbits. The half-life of the compound LM-
1554 was also found to be very small (64-70 mins). The smaller half-life of the compound suggests that the compound has either poor bioavailability and/or fast clearance (Benet et al., 1996, Gibaldi and Perrier, 1982). The clearance rate for the compound LM-1554 was found to be very low, both in dogs (Table-15), and in rabbits (Table-16). Smaller half-life may be due to poor bioavailability or rapid intestinal metabolism (Gibaldi and Perrier, 1982). The volume of distribution (Vd) of the compound LM-1554 was found to be very less (< 2 l/kg). The lower Vd value suggests that the compound LM-1554 was not being deposited in any particular tissues (Benet and Galazzi, 1979; Benet, 1983). If a compound is poorly absorbed from the gastrointestinal tract and is not being deposited in any particular tissues, but still produces antihyperlipaemic effect strongly suggests the gastrointestinal tract to be the main site of action of the drug.

The second set of evidences favouring gastrointestinal tracts as the site of action is the results of pharmacodynamic study which indicate that the compound LM-1554 reduced the serum lipid levels when given orally and not intraperitoneally. It was found that the compound LM-1554 when administered orally, not only prevented cholesterol-induced hyperlipidaemia (Fig.-71-73), but also reversed the raised lipid levels in cholesterol-fed rats (Fig.-75-78). The results of these studies were less than that for gemfibrozil at the same dose level (Table-20). It is not clear whether the systemic availability of LM-1554 plays any role for its antihyperlipaemic activity. Intraperitoneal administration of LM-1554 did not show any antihyperlipaemic activity.

Third piece of evidence comes from the results of Quantitative Structure Activity Relationship (QSAR). In QSAR using information about structures and their pharmacological activities of a relatively large group of congeners, it is possible to identify the properties required for optimal action. Recent advances in computational chemistry and structural analysis of organic compounds have given new impetus to the quantitation of structure activity relationship and drug design (Ross, 1990b). This development of new description of molecule and more powerful correlative methods have the ability to throw some light on mechanism of action of new compounds, as well as give idea about the substitution required to develop most potent compounds without synthesising them.

In the present investigation, two series of LM-1554 analogues (2-substituted methylbenzo(b)thieno(3,2-d)pyrimidin-4(3H)-ones and 2-substituted methyl 7,9,-
dimethylpyrido (3',2' :4,5)thieno(3,2-d)pyrimidin-4(3H)-one) were synthesised. These compounds were screened for biological activity. Various physicochemical parameters like electronic (δppm, σ*), lipophilic (logk', f), stearic (MR, ki) parameters were determined for all compounds. The biological activity was correlated with physicochemical parameters by stepwise single and multiple regression analysis. A perusal of the equations derived from the regression analysis (eq. 7-10), indicates a positive and significant correlation only between the biological activity and electronic parameters (r\geq 0.08). The positive sign of the coefficient of the electronic parameters (σ* and δppm) indicates the positive influence of the electron-withdrawing substituents in increasing the biological activity.

Antihyperlipaemic agents like cholestyramine and colestipol produces their effect by exchanging chloride ion for the negatively-charged bile acid (Moore et al., 1986). In the present investigation, substitution with higher electronic negativity in thienopyrimidine compounds was associated with increase in potency of antihyperlipaemic activity. The lipophilic and stearic parameters did not appear to be significantly correlated with biological activity. All these results further suggest that at the gastrointestinal site, the compound LM-1554 causes the antihyperlipaemic activity.

The absorption of lipids occurs from the gastrointestinal tract and the absorbed lipids are transported to the liver via portal vein. In the liver, they undergo metabolism and there, endogenous lipids also come into the picture (Bilhemeir, 1986). Hence, the alteration in cholesterol level in portal vein may give a picture of the effect of a drug on cholesterol absorption. As a direct evidence, to substantiate the mechanism of action of LM-1554, we studied the effects of the compound on the cholesterol levels in the blood collected from the portal vein. The cholesterol levels in the blood collected from the portal vein were found to be significantly higher as compared to controls after three hours of cholesterol administration to rats. This increase in cholesterol level was significantly prevented when the compound LM-1554 was administered simultaneously.

In conclusion, the compound LM-1554 was found to be effective as an antihyperlipaemic agent and has a potential to be developed as an antihyperlipaemic agent. The mechanism of action of the compound LM-1554 appears to inhibit cholesterol absorption of gastrointestinal level.
PART-2

LM-2616: A $\beta_1$-ADRENOCEPTOR ANTAGONIST WITH $\beta_2$-ADRENOCEPTOR AGONIST EFFECT

The compound LM-2616 was found to specifically inhibit the adrenaline and isoprenaline-induced positive chronotropic effects (Fig.-80,82). The isoprenaline-induced hypotensive response was, however, found to be potentiated in dogs (Fig.-83). These results indicate the presence of two different populations of $\beta$-adrenoceptors, one mediating the chronotropic responses and the other mediating inotropic responses. It appears that the inhibition of positive chronotropic actions of adrenaline and isoprenaline is through the sub-type of $\beta_1$-adrenoceptors mediating chronotropic effect and the potentiation of isoprenaline-induced hypotensive effect is mediated through $\beta_2$-adrenoceptors. The presence of two different types of beta-adrenoceptors have also been reported in 1977 by Bonelli (1977)

The results of the effect of LM-2616 in guinea-pig heart also support these findings. In this preparation, the compound LM-2616 inhibited the isoprenaline-induced positive chronotropic responses (Fig.-92). However, the isoprenaline-induced positive inotropic effect was potentiated by the compound LM-2616 (Fig.-93).

The antagonism of adrenaline- and isoprenaline-induced chronotropic responses by the compound LM-2616 suggests its possible usefulness in cardiac arrhythmias. Propranolol, a non-selective beta-adrenoceptor antagonist is very commonly used in cardiac arrhythmias. It is not uncommon that many of the beta-adrenoceptors blockers possess local anaesthetic activity. This activity is attributed mainly to membrane-stabilising activity and is specifically beneficial for anti-arrhythmic activity (Black and Prichard, 1973; Shand, 1975). The compound LM-2616 was also found to produce 100% local anaesthetic effect in two types of model i.e. surface anaesthetic model and infiltration anaesthetic model. The local anaesthetic activity of the compound was comparable to the lignocaine (Table-31,32)

Recently it has been reported by Kaumann and Lemoine (1987) and Bristow et
al.(1986) that not only β₁-adrenoceptors but also β₂-adrenoceptors contributes to the increased contractile force of human myocardium. β₂-Adrenoceptors have been identified in the heart of dog (Einstein et al., 1979) and of man (Brodde et al.; 1983; Wilson, 1984). These cardiac adrenoceptors mediate the positive inotropic responses (Molenaar and summers, 1987).

In guinea pig heart, terbutaline, a specific β₂-adrenoceptor agonist produced a dose dependent positive inotropic effect which was competitively blocked by butoxamine, a specific β₂-adrenoceptor antagonist (Fig.-94). The pA₂ values of butoxamine for this preparation was found to be 9.18. The pA₂ values of butoxamine against terbutaline were found to be similar in the rat uterus and guinea pig tracheal chain preparations (Table-30). Thus our results also indicate the presence of β₂-adrenoceptors in the heart which are responsible for inotropic effect. The pA₂ values of butoxamine against LM-2616 were also found identical to terbutaline in guinea pig tracheal chain and rat uterus preparations (Table-30).

The presence of β₂-adrenoceptors in rat uterus has been reported by Tothill (1967). Further evidence for β₂-adrenoceptor agonistic activity for the compound LM-2616 was obtained from the results of rat uterus where the compound LM-2616 per se produced a dose- dependent relaxation. This was not blocked by practolol, a specific β₁-adrenoceptor antagonist, but was blocked competitively by propranolol, a non-specific β-adrenoceptor antagonist and butoxamine, a specific β₂-adrenoceptor antagonist (Fig.-86,88). Terbutaline, a specific β₂-adrenoceptor agonist also produced a dose-dependent relaxation of depolarised rat uterus and this was blocked by butoxamine (Fig.-87). The pA₂ value of butoxamine was found to be identical when tested against the compound LM-2616 or terbutaline (Table-30). Similar results were obtained on guinea pig tracheal chain (Fig.-89,90). Isoprenaline produced a dose dependent relaxation of guinea pig tracheal chain. The responses to isoprenaline were potentiated by the compound LM-2616 without affecting maxima (Fig.-91).

Preliminary toxicological studies indicate that the compound LM-2616 is safe, as it has the median lethal dose (LD50) 480 mg/kg.

A number of β-adrenergic antagonist are available as therapeutic agents for the treatment of angina pectoris, cardiac arrhythmias, hypertension, migraine, thyrotoxicosis, anxiety, tremors glucose, and myocardial infarct (Weiner, 1985: 205).
Differences among the various agents are related not only to their variable selectivity for $\beta_1$ and $\beta_2$ receptors, but also to their non-$\beta$-blocking properties, such as intrinsic sympathomimetic activity, membrane stabilising effects, lipophilicity, metabolism and duration of action. All the agents available for clinical use, however, competitively, antagonise the $\beta$-receptor-mediated effects of sympathomimetic amines due to a direct interaction with the receptor that is independent of any effects on adrenergic nerves or on the basic response mechanism of effector cells. Although the major action of these agents is to block $\beta$-receptors, some (e.g., pindolol, oxprenolol, acebutolol, practolol and alprenolol) produce weak agonistic activity. This partial agonist activity or ISA may be of advantages in patients at risk from $\beta$-blockade, for example, asthmatic and subjects with congestive heart failure, because the ISA may limit the degree of $\beta$-receptor antagonism. In fact, a combination of $\beta_1$-adrenoceptor antagonist and $\beta_2$ adrenoceptor agonist have been shown to be ideal in heart failure (Mugge et al., 1985; Zerkowski et al., 1986). Reduction of afterload by means of vasodilation has been shown to offer a useful therapeutic alternative to inotropic stimulation in treatment of heart failure, allowing myocardial oxygen consumption to be lowered, despite an increased cardiac output (Weber et al., 1982). Also, there is a selective down regulator of $\beta_1$-adrenoceptors, associated with high sympathetic drive in the failing heart (Cohn et al., 1984; Bristow et al., 1982). The $\beta_2$ - adrenoceptors of heart are relatively resistant to down regulation in heart failure (Bristow et al., 1986). Thus, $\beta_2$ - adrenoceptor agonists have a therapeutic potential in the heart failure.

In heart failure, increase in sympathetic tone gives rise to useful inotropic stimulation, but may result in excessive tachycardia and arrhythmia. The $\beta_1$-adrenoceptor antagonistic activity can be useful to prevent this tachycardia. Similarly, also during quinidine therapy for atrial flutter, digitalisation of patient is often necessary (Bigger and Hoffman, 1980).

$\beta$-Adrenoceptor blocking agents that possess ISA or are $\beta_1$-selective may also be desirable for use in patients with peripheral vascular disease, such as intermittent claudication or Raynaud's phenomenon. In these patients, blockade of vasodilatory $\beta_2$-receptors may further reduce blood flow, which is already compromised in the extremities. Other side effects of $\beta$-blockers include a number of metabolic and central nervous system effects. The metabolic effects are mainly due to their $\beta$-blocking activity and include hypoglycemia in insulin-dependent
diabetes, a masking of the symptoms of hypoglycemia and in certain circumstances, hyperkalemia. These effects are due to blockade of β-receptor mediated insulin release, hepatic and skeletal muscle $K^+$ uptake and increases in cardiac rate and force of contraction. β-Blockers, particularly non-selective agents, may also impair lipoprotein metabolism, leading to increases in serum triglycerides and decreases in high-density lipoprotein cholesterol level.

Beta-blockers are also widely used as antihypertensive drugs for the last twenty-five years. But Milne and Buckley (1991) reported a new compound, celiprolol, which is a third-generation, cardioselective, beta-adrenoceptor blocking agent. They appear to be safe and effective alternatives to currently available beta-blockers for antihypertensive effect. These agents also possess weak vasodilating and bronchodilating effects attributed to partial, selective beta$_2$-adrenoceptor agonistic activity. They have certain advantages over conventional beta-blockers. They also reduce triglycerides, LDL cholesterol and total cholesterol in some patients. Blood glucose levels and insulin requirements are not significantly altered in Type-1 diabetics.

In the present investigation, the compound LM-2616 was found to possess β$_1$-adrenoceptor antagonistic activity with β$_2$-adrenoceptor agonistic activity. Thus, it has potential to be developed as an antiarrhythmic and/or a third-generation antihypertensive agent.

**STRUCTURE ACTIVITY RELATIONSHIP OF ANALOGUES OF THE COMPOUND LM-2616**

The affinity of a drug for its receptor and its intrinsic activity are intimately related to its chemical structure. The relationship is many a times quite stringent. Relatively minor modifications in drug molecule, including such subtle changes as stereoisomerism, may result in major changes in pharmacological properties. Exploitation of Structure-Activity-Relationship (SAR), has on many occasions, led to the synthesis of valuable therapeutic agents. With adequate information about both, the molecular structures and the pharmacological activities of a relatively large group of congeners, it is possible to identify those properties which are required for optimal action, such as receptor size, shape, position and orientation of charged group or hydrogen bond donors and so on (Ross, 1990b).
In elucidating the SAR of β-adrenergic agonist, it has been found that they belong to two major chemical classes. the phenethanolamines and the aryloxypropanolamines. This last class of compounds has only recently been determined as having agonist activity. Structural requirements have been summarised by Mimnaugh (1986). As originally observed by Barger and Dale, a basic amine is essential for agonist activity. Konzett demonstrated that the size of the amino alkyl substituent is important for differentiating β-agonist and alpha-agonist activity. Bulky substitution on amino group increases β-adrenergic activity.

A considerable number of analogues have been synthesised in which catechol nucleus of epihephrine has been modified. Elimination of one or both of hydroxyl groups results in substantial or complete loss of activity.

If single phenolic hydroxyl group is present, its position on ring is important for selectivity. Thus, the meta hydroxyl in phenylephrine provides part of alpha-receptor agonist selectivity of this compound, while para hydroxyl group in isoxuprine, nylidrin and salbutamol contribute to their activity as β-receptor selective agonists. In general, the substitution of any group for a phenolic hydroxyl on an adrenergic agonist will convert that agonist to an antagonist. Another variation in aromatic nucleus which appears to favour bronchial β₂-agonist activity is 3,5-dihydroxyl substitution pattern shown in terbutaline and metaproterenol. Many types of heterocyclic rings have been substituted for benzene ring. With few exceptions, the substitutions have resulted in loss of agonist activity.

Early studies showed that the two carbon atom distance between aromatic ring and nitrogen is important for optimal binding to receptor. Recently it has been shown that an oxypropanolamine side chain too possesses agonist activity. Substitution on α-carbon generally leads to decreased agonist activity, but α-methyl substitution allows for more efficient uptake of compound from the synaptic cleft. Presence of β-OH group is considered essential for full agonist activity. Further, keeping this substitution in basic molecule and substituting hydroxyl group with chloro group resulted in compound having β-adrenoceptor antagonistic activity. Removal of hydroxyl group from β-carbon of ethylamine side chain produces loss of agonistic activity. Ether linkage between aromatic and amino side chain renders compound β-adrenoceptor antagonist for e.g. oxypropylamino compounds like practalol, propranolol, acebutolol. The results obtained from the screening of various analogues of compound LM-2616 (M1 to M12) provide a good structure activity relationship for the activity on beta-adrenoceptors. There is no
structural resemblance of the compound LM-2616 and its analogues with any known β-adrenergic compound. However, bulky substitution at amino group at 4-position appears to be important for β-adrenergic activity. Substitution of various amines at 4-position of parent molecule has resulted in compound with differential activity at β₁ -and β₂ -adrenoceptor.

Simple secondary amine [diethylamine (M10)] at 4-position of parent molecule showed potent β₁ -adrenoceptor antagonistic as well as β₂ -adrenoceptor agonistic activity. Cyclic secondary amine at 4-position [e.g. piperidine (M13)] showed both activities i.e. β₁ -antagonistic and β₂ -adrenoceptor agonistic but lesser than secondary amine (M10). Aliphatic cyclic primary amine [cyclohexamine (M7)] also shown β₁ -adrenoceptor antagonistic activity with β₂ -agonistic activity but also lesser than secondary amine (M10). Incorporation of secondary nitrogen in secondary amine [e.g. N-methyl piperazine (LM-2616)] showed good activity as β₁ -adrenoceptor antagonist and β₂ -adrenoceptor agonist. Increase in lipophilicity of piperazine [e.g. N-methyl (LM-2616), N-furoyl (M4) and N-benzoyl (M3)] resulted in increase in both the activities but again the activity is lesser than M10. Thus for β₁ -adrenoceptor antagonistic with β₂ -adrenoceptor agonistic activity, substitution at 4-position with simple secondary amine is advisable.

Increase in carbon chain of amine at 4-position [e.g. Novaldamine (M1), N,N-diethyl amino propylamine (M6)] showed increase in β₁ -adrenoceptor antagonistic activity over β₂ -adrenoceptor agonist activity. This shows that longer the carbon chain greater is the β₁ - antagonist activity. However, aromatic amine at 4-position (M2, M11, M12) produces increase in β₂ -agonistic activity over β₁ -antagonistic activity. Among the aromatic amino substituted compounds, it is clearly observable that the activity is governed by steric factor (M2 > M11 > M12). Sterically hindered aromatic amine [e.g. 2,6-xylidine (M2)] was found more potent than other aromatic amines (M2 > M11 > M12).

It was also planned to check the importance of pyridothiphene ring attached to pyrimidine on biological activity. Compounds with highly lipophilic ring, cyclohexyl thiophene ring (M9), was found less active than LM-2616. Benzothiophene, a bioester of LM-2616 (M5) was found more active as β₁ -antagonist with β₂ -agonostic activity than LM-2616. Compound with 2,3-dimehtyl thiophene ring (M8), lowest lipophilic compound was found the most active as than any other compound. These observations confirm that as the lipophilicity of the ring attached
to pyrimidine increases, the activity decreases.

Among thirteen compounds studied M8 appears to be a potent $\beta_1$-adrenoceptor antagonist with $\beta_2$-adrenoceptor agonist properties.