Introduction
INTRODUCTION:

Somatostatin, a cyclic tetradecapeptide, is a physiological regulator of growth hormone (GH) secretion (Brazeau et al., 1972) that is also known to inhibit the secretion of multiple hormones such as insulin, glucagon, thyroid stimulating hormone etc. (Dutta, 1993). The anti-secretory and anti-proliferative activity of somatostatin has generated a lot of interest in the biological activity of this neuropeptide (Pollak and Schally, 1998; Hofland et al., 1994; Gillispie et al., 1998). The therapeutic potential of somatostatin, however, remains unfulfilled due to its short serum half life (plasma half life = 1 min.). This has led to the development of highly potent somatostatin analogs with increased plasma half life. Three cyclooctapeptide analogs, namely RC-160, octreotide and BIM23014 exhibit a marked increased stability in circulation (plasma half life 2 hr.) and are atleast 50 times more potent than somatostatin in inhibiting GH secretion in vivo (Dutta, 1993).

RC-160 is one of the most potent and extensively studied agonists of somatostatin. The anti-proliferative and anti-secretory activity of RC-160 has been demonstrated in a number of experimental models of pancreatic, prostate, renal, hepatocellular and mammary cancers (Kournamalis et al., 1998; Qin et al., 1995; Jungwirth et al., 1998). RC-160 has been reported to be 500-times more potent than octreotide or BIM23014 in the inhibition of GH release in vitro (Hofland et al., 1994). RC-160 has also been found to potently inhibit EGF-induced proliferation of human cancer cells in vitro (Liebow et al., 1989).

The activity of somatostatin is mediated through high affinity, G-protein coupled receptors on target cells (Patel et al., 1997). These receptors belong to five distinct subtypes (SSTRx, x=1-5). The inhibitory effect of RC-160 on tumor growth may be mediated directly by SSTR's on cancer cells, or induced indirectly by the inhibition of growth factors such as IGF-I, EGF, insulin etc. (Liebow et al., 1989; Catteneo et al., 1996). The anti-neoplastic effect of RC-160 may also be attributed to its ability to inhibit tumor angiogenesis (Barrie et al., 1993; Woltering et al., 1997).
The delivery of therapeutic peptides to the site of action remains a major challenge even today. Although, somatostatin analogs like octreotide and RC-160 are longer acting than the native hormone, their biological half life of less than 2 hours requires either frequent administration or sustained release formulation, to attain enhanced anti-proliferative activity and a long term suppression of hormones like GH and IGF-1 (Lamberts et al., 1995; Rothen-Weinhold et al., 1999; Dutta, 1993). Pharmacokinetic profiles of long-acting sustained release formulations for octreotide (Sandostatin™ LAR) and lantreotide (Somatuline™ LP) have been extensively studied (Grass et al., 1995; Johnson et al., 1995). Even though both formulations are able to modulate GH release, neither can be considered optimal in view of an “initial burst” of drug release in one case (Somatuline) and an initial delay in peptide release in the other (Sandostatin). The administration of these anti-tumor agents is also limited by their pleotropic nature and side effects like inhibition of gall bladder emptying which leads to increased incidence of cholesterol gallstones (Lamberts et al., 1996). The acute administration of somatostatin has been found to produce receptor desensitization which results in diminished therapeutic response and induction of tolerance (Lamberts et al., 1996). Hence, there is a need for further modification of somatostatin analogs to endow them with improved stability, receptor affinity, selectivity and better delivery properties.

Lipophilization of neuropeptides represents an alternate strategy to increase their stability, bioavailability, absorbability, as well as their permeability through biomembranes. Unlike sustained release formulations, lipophilization can also confer peptides with enhanced receptor selectivity. The attachment of fatty acids to peptides like VIP led to the generation of highly potent and selective VIP antagonists (Gozes et al., 1995; Gozes et al., 1996) which have provided novel tools in drug design for neurodegenerative disorders like Alzheimer’s disease or disturbances in biological rhythms.

The first objective of the present study, was to evaluate the effect of lipophilization (conjugation of fatty acyl moiety) on the anti-neoplastic activity of RC-160, and to
determine whether such derivatization of RC-160 could increase its stability and anti-proliferative activity. The anti-proliferative activity of RC-160 and the lipopeptides was evaluated on a variety of human cancer cell lines in vitro, with particular emphasis on human oral carcinoma. The effect of varying carbon chain length on the activity of lipophilized RC-160 was also studied. The study also aimed to obtain an insight into the selectivity, permeability of these lipopeptides, as well as their ability to inhibit tumor angiogenesis in vitro, relative to RC-160.

The second objective of this study was to design and synthesize chimeric peptides composed of Growth Hormone Releasing Peptide (GHRP-6) and the biologically active fragment of somatostatin. The total number of somatostatin analogs is greater than 450. However, all these analogs are agonists of somatostatin; very few antagonists of somatostatin had been described (Bass et al., 1996). Since, GHRP-6 is the functional antagonist of somatostatin, we conjectured that these chimeric peptides might function as somatostatin antagonists. The biological activity of these chimeric peptides was ascertained by their ability to induce/inhibit GH secretion, in rat pituitary adenoma cell line, GH3.
AIMS AND OBJECTIVES:

The neuropeptide somatostatin is known to play a very important role in the endocrine, autocrine and paracrine functions in living organisms (Schofl et al., 1994). The somatostatin analog RC-160 has been extensively studied for its anti-proliferative and anti-secretory activity (Pollak and Schally, 1998). Despite the very high potency and specificity of RC-160, its application as a clinically useful drug has been problematic because of its short biological half life and serious delivery problems (Dutta, 1993).

As discussed previously lipophilization of bioactive peptides, is one of the strategies to improve their stability, bioactivity and permeability across biomembranes (Gozes et al., 1995, 1996). In the light of the above observations, the objectives of the present study were:

1. To synthesize, purify and characterize lipophilized derivatives of RC-160, of varying carbon chain lengths (chap. 1).

2. To evaluate the effect of lipophilization (conjugation of fatty acyl moiety), on the anti-neoplastic activity of RC-160, and to determine whether such derivatization of RC-160 could increase its anti-proliferative activity (chap. 2, 3 and 4) and anti-angiogenic activity (chap. 3). The anti-proliferative activity of RC-160 and the lipopeptides was evaluated on a variety of human cancer cell lines in vitro (chap. 2, 3 and 4), with particular emphasis on human oral carcinoma (chap. 4). The signaling pathways mediating the anti-proliferative activity of RC-160 and the lipopeptides in oral carcinoma were also studied (chap. 4). The effect of varying carbon chain length on the bioactivity of lipophilized RC-160 was also studied (chap. 2, 3 and 4).

3. To evaluate the effect of lipophilization, on the GH-inhibitory activity of RC-160 (chap. 5: part A). The effect of varying carbon chain length on the ability of lipophilized RC-160 to inhibit GH secretion from rat pituitary adenoma cells, relative to RC-160 was studied (chap. 5: part A).
4. To design and synthesize chimeric peptides composed of Growth Hormone Releasing Peptide (GHRP-6) and the biologically active fragment of somatostatin. The GH-inhibitory activity of these peptides was determined in vitro, using the rat pituitary adenoma cell line GH3 (chap. 5: part B).

5. To evaluate the relative stability of lipophilized RC-160 in serum and in the presence of crude and purified proteases in vitro (chap. 6).