Oral cancer defines malignancy of the lip, mouth or tongue. Histologically, it is predominantly a squamous cell carcinoma. It is the sixth most common cancer worldwide, and its relative survival rates are amongst the lowest of all cancers. In India, oral cancer comprises about 40% of the total reported cancer cases. Tobacco-chewing, consumption of alcohol, areca nut alkaloids, viruses like EBV and HPV contribute towards the development of malignancy of oral cancers. Oral carcinoma is characterized by poor prognosis and treatment. Standard therapy for patients with oral carcinoma is surgery or radiation therapy or a combination of both (Christensen, 1998). However, successful therapy for patients with advanced disease is often accompanied by severe dysfunction and disfigurement (Kaur and Fee, 1993).

Previous studies have demonstrated that the growth and transformation of the oral epithelium is regulated by several polypeptide hormones and growth factors. Amongst these, the epidermal growth factor (EGF) appears to be predominantly involved in the growth and proliferation of the oral epithelium (Christensen, 1998). The overexpression of high affinity EGF receptors in malignant oral carcinomas, has implicated this growth factor in the neoplastic transformation of oral epithelium (Christensen, 1992; Bergler et al., 1989). These findings suggest that new approaches to the treatment of oral cancers may be based on the functional manipulation of EGF activity, using peptide analogs, such as synthetic agonists of somatostatin (Liebow et al., 1989).

Somatostatin is a regulatory neuropeptide with a wide range of physiological and pharmacological effects (Gilles, 1997). The anti-neoplastic activity of RC-160, has been extensively discussed earlier (refer to review of literature) and is mediated through high affinity, G-protein coupled somatostatin receptors (SSTRs) on target cells (Patel et al., 1997). It has been suggested that the inhibitory effect of RC-160 on tumor growth may be mediated directly by SSTRs on cancer cells, or induced indirectly by the inhibition of growth factors such as EGF, IGF-1 etc. (Liebow et al., 1989; Pollak and Schally, 1998).
The anti-neoplastic activity of RC-160 has also been shown to be mediated through the activation of tyrosine phosphatase, which causes the dephosphorylation of EGF-induced tyrosine kinase. The EGFR family consists of proteins having intracellular, extracellular, and transmembrane domains. Three of these domains act as: a tyrosine kinase enzyme, a regulatory site for ligand binding, and a transducing element for conformational change. The intracellular domain of the EGF receptor (which is overexpressed in oral carcinoma), possesses tyrosine kinase activity. The binding of EGF to its receptor also activates intracellular tyrosine kinases, which mediate the signal for increased cell growth (Volberg et al., 1997). The downregulation of tyrosine kinase using specific tyrosine kinase inhibitors (Christensen, 1998) or monoclonal antibodies against EGFR (Modjtahedi et al., 1993) are some of the extensively studied approaches to suppress cell growth in oral cancers. The neuropeptide somatostatin has been found to be a potent inhibitor of EGF-stimulated cell proliferation, hence somatostatin agonists may be of potential importance in the therapy of oral carcinoma. However, few studies have addressed the anti-proliferative activity and downstream signal transduction pathways of somatostatin agonists in oral carcinoma.

One of the objectives of the present study was to investigate whether RC-160 could inhibit EGF-induced proliferation, in the oral carcinoma cell line KB; and whether such an inhibition was mediated by the downregulation of tyrosine kinase activity in KB cells. The present work also investigates the status of somatostatin receptor expression on KB cells. This study also attempted to ascertain whether the anti-proliferative activity of RC-160 was mediated by somatostatin receptors on KB cells.

The synthesis of lipophilized derivatives of RC-160 has already been described earlier (refer chap.1, pp. 35-37). The anti-proliferative activity of these lipopeptides were determined relative to RC-160, in the human nasopharyngeal cell line KB. Another important objective of the present study was to determine whether the
lipopeptides, were similar to RC-160. The receptor selectivity of lipophilized RC-160, as well as its ability to inhibit tyrosine kinase activity and intracellular cAMP levels, relative to RC-160 in the oral cancer cell line KB in vitro, was also compared.

**MATERIALS AND METHODS:**

**Peptide Synthesis:**
Peptide synthesis was carried out according to the manual Fmoc solid phase strategy, as described in chap. 1, pp. 35-37.

**Cell Culture:**
The human oral carcinoma cell line KB was obtained from National Center of Cell Science Pune, India. It was maintained in exponential growth in RPMI 1640 medium supplemented with 2mM glutamine, 2.2g/L sodium bicarbonate, 25mM HEPES, 100units/ml penicillin, 50μg/ml streptomycin, 40μg/ml gentamycin and 10% FCS, at 37°C in a humidified atmosphere containing 5% CO₂ in air.

**Proliferation Assay:**
KB cells cultured to 70% confluence were harvested using 0.05% trypsin-2mM EDTA solution and replated in medium supplemented with 10% FCS, in 96 well microtiter plates at a density of 5000 cells/well. The plates were incubated for 36 hours to allow complete reattachment of the cells. Subsequently cells were incubated with serum free medium for 36 hours to unmask the effect of endogenous growth factors. Thereafter, cells were stimulated with 0.16μM EGF, in the presence or absence of peptide analogs for 36 hours. The concentration of EGF to be used for the experiment was ascertained by a proliferation assay done previously. The analogs were added every 12 hours, during the 36 hour incubation (Catteneo et al., 1996).

Studies using inhibitors like sodium orthovanadate, okadaic acid were done by incubating the cells with these inhibitors 4 hours prior to the addition of EGF alone or with EGF and the peptide (Buscail et al., 1994, 1995). The assay was carried out
as described above for 36 hours. Studies using pertussis toxin were done by incubating the quiescent KB cells with 50ng/ml pertussis toxin, for 18 hours prior to the commencement of the assay.

$^3$H Thymidine (1μCi/well; specific activity 2 Ci/mmol, DU PONT NEN, U.S.A.) was added during the last 6 hours of incubation. The cells were harvested and collected on glass fiber filters using an automatic cell harvester (Skatron, Norway). The radioactivity incorporated was measured using a β-counter (Wallac, Finland).

**Receptor Binding Assay:**

Receptor binding assays were performed as discussed previously in chap. 2, pp. 42-43. Studies to determine the effect of 100μM GTPyS and 50ng/ml pertussis toxin on the binding of $^{125}$I-[Tyr$^1$]-somatostatin-14 to somatostatin receptors on KB cells were done as previously described (chap. 3, page 53).

**Tyrosine Kinase Assay:**

Phosphorylation of a broad specificity peptide substrate was performed using a nonradioactive tyrosine assay kit (Boehringer Mannheim, Germany). $1\times10^6$ cells were treated with 0.16μM EGF for about 15 minute in the presence or absence of somatostatin analogs. The cells were lysed and the tyrosine kinase activity was determined measuring the phosphorylation of a broad specificity peptide, as per the manufacturer's instructions.

**Measurement of intracellular cAMP:**

KB cells were cultured in 24-well plates at a density of $5\times10^4$ cells/well, in RPMI-1640 containing 10% FCS, until they became 70 to 80% confluent. Cells were incubated for 30 minutes at 37°C in an assay buffer consisting of 25mM Tris-acetate (pH=7.4), 0.25mM sucrose, 0.5% BSA and 0.5mM 3-isobutyl-1-methylxanthine (MIBX), in the presence or absence of RC-160 or lipophilized RC-160 and 0.16μM EGF or a combination of both (Qin et al., 1995). The reaction was terminated by
adding ice cold lysis buffer to each well. The concentrations of cAMP in the cellular lysate were determined using cAMP RIA kit (DU PONT NEN, U.S.A), according to the manufacturer's instructions.

**Statistical analysis:**
All data are expressed as the mean ± SEM. Mean values between the treatment group and the control group were analyzed by using ANOVA (Micro Cal Origin Ver. 2.0). Data were considered significant when the value of p was less than 0.05.

**RESULTS:**

**Anti-proliferative activity of RC-160 and lipopeptides:**
EGF was found to stimulate the proliferation of KB cells *in vitro* (Fig. 16). The maximal stimulation of the growth of oral carcinoma cells was found to be at 0.16μM of EGF.

RC-160 was found to suppress EGF-induced proliferation of human oral carcinoma cells *in vitro*. The dose response graph was found to be biphasic, the maximal suppression of cell growth was obtained at 10ng/ml of RC-160 (p<0.01) in KB (Fig. 17A). Fig. 17B, shows the relative anti-proliferative activities of lipophilized derivatives of RC-160. In this graph the concentration of the peptide displaying the maximum anti-proliferative activity (marked by an asterisk in Fig. 17A) has been plotted against the carbon chain length of the lipopeptide. The anti-proliferative activity of all other lipophilized derivatives of RC-160 except butanoyl-RC-160, towards KB cells, are equivalent to each other. The lipopeptides (except butanoyl-RC-160) induce a similar suppression of cell growth as RC-160, at a 10-fold lower concentration. As seen with cell lines studied previously, the attachment of butanoic acid to RC-160 seems to lower its anti-neoplastic activity. The anti-proliferative activity of butanoyl-RC-160, is exhibited at a 10-fold higher concentration as compared to RC-160 (p<0.01).
Fig. 16 EGF-induced proliferation of the human oral carcinoma cell line KB \textit{in vitro}.
Fig. 17 Antiproliferative activity of RC-160 and its lipophilized derivatives, on the human oral carcinoma cell line KB.

* Conc. of the peptide showing maximum antiproliferative activity.
Fig. 17 Anti-proliferative activity of RC-160 and its lipophilized derivatives, in KB cells. The concentration of the lipopeptide showing the maximum anti-proliferative activity (marked by an asterisk in Fig. 17 A) is plotted versus the carbon chain length of the lipopeptide.
Receptor Binding Assay:
The binding of $^{125}$I-[Tyr$^1$]-somatostatin-14 was studied in the human oral cancer cell line KB. Scatchard analysis indicated the presence of two distinct classes of specific binding sites on KB (Fig. 18). The $K_d$ and $B_{max}$ values for each class of binding sites, is shown in Table 8A. RC-160, butanoyl-RC-160 as well as its myristoylated counterpart could effectively displace $^{125}$I-[Tyr$^1$]-somatostatin-14, bound to the oral carcinoma cells, in a dose dependent manner (Fig. 19). The concentration of peptide which causes a 50% inhibition of binding of $^{125}$I-[Tyr$^1$]-somatostatin-14 to the above cell lines (IC$_{50}$) is shown in Table 8B. The IC$_{50}$ of myristoyl RC-160 is not significantly different from RC-160. However, the IC$_{50}$ of butanoyl-RC-160 is significantly higher than RC-160 itself (Table 8B) ($p<0.001$).

The anti-proliferative effect of lipophilized RC-160, in vitro seems to be mediated by the presence of specific somatostatin receptors on KB cells (Fig. 18). GTP$_\gamma$S was able to significantly block the binding of $^{125}$I-[Tyr$^1$]-somatostatin to the receptor (Fig. 20) ($p<0.001$). However, pertussis toxin did not cause the dissociation of $^{125}$I-[Tyr$^1$]-somatostatin from its receptors on KB cells (Fig. 20). This suggests that RC-160 as well as its lipophilized derivatives, mediate their anti-proliferative activity through pertussis toxin insensitive G-proteins.

Signal transduction pathways underlying the anti-proliferative activity of lipophilized RC-160:
RC-160 and its lipophilized derivatives potently inhibited EGF-stimulated proliferation of KB cells in vitro (Fig. 17B). The anti-proliferative activity of RC-160 and lipopeptides, was not abrogated by 100$\mu$M sodium orthovanadate (tyrosine phosphatase inhibitor), 1.25 nM okadaic acid (inhibitor of serine/threonine phosphatase 1A and 2A) and 50ng/ml pertussis toxin (Fig. 21A-C).
Fig. 18 Scatchard Analysis indicates the presence of two distinct binding sites on the human oral carcinoma cell line KB.
Fig. 19 Displacement of bound $^{125}$I-[Tyr$^1$]-somatostatin from KB cells by RC-160 and its lipophilized derivatives
Table 8A: Specific binding of $^{125}$I-[Tyr$^1$]-somatostatin to KB cells in vitro. The cells were incubated with 0.5nM of $^{125}$I-[Tyr$^1$]-somatostatin. Scatchard analysis was used to determine the nature of binding sites as well the $B_{max}$ values.

<table>
<thead>
<tr>
<th>SNo.</th>
<th>CELL LINE</th>
<th>$K_d$ (nM)</th>
<th>$B_{max}$/5x$10^4$ cells (fmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>KB</td>
<td>0.641 (l*)</td>
<td>54.1 (h*)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.002 (h*)</td>
<td>0.8 (h*)</td>
</tr>
</tbody>
</table>

l*: low affinity  
h*: high affinity

Table 8B: Displacement of $^{125}$I-[Tyr$^1$]-somatostatin binding by lipopeptides and RC-160. The KB cells were incubated with 0.5nM of $^{125}$I-[Tyr$^1$]-somatostatin in the presence of the above peptides, for 2 hours at 4°C. The table below shows the IC$_{50}$ values for the peptides.

<table>
<thead>
<tr>
<th>SNo.</th>
<th>LIPOPEPTIDES</th>
<th>KB IC$_{50}$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>RC-160</td>
<td>0.29</td>
</tr>
<tr>
<td>2)</td>
<td>Butanoyl-RC-160</td>
<td>2.16</td>
</tr>
<tr>
<td>3)</td>
<td>Myristoyl-RC-160</td>
<td>0.34</td>
</tr>
</tbody>
</table>
Fig. 20 Abrogation in the binding of $^{125}$I-[Tyr$^1$]-somatostatin in the presence of 100μM GTP$\gamma$S and 50ng/ml pertussis toxin to somatostatin receptors on KB cells.
Fig. 21 Effect of pertussis toxin (PT) on the anti-proliferative activity of RC-160 and its lipophilized derivatives on the oral carcinoma cell line KB. The peptides were used at the concentration, at which they displayed the maximum anti-proliferative activity on KB cells (Fig. 17B).
Fig. 21 Effect of the tyrosine phosphatase inhibitor sodium orthovanadate on the antiproliferative activity of RC-160 & its lipophilized derivatives on the human oral cancer cell line KB. The concentration of the peptides used were those at which they displayed the maximum anti-proliferative activity, in KB cells (Fig. 17B).
Fig. 21 Effect of Ser/Thr phosphatase inhibitor okadaic acid on the anti-proliferative activity of RC-160 & its lipophilized derivatives on the oral cancer cell line KB.
However, it was found that RC-160 as well as the lipopeptides could potently inhibit EGF-stimulated protein tyrosine kinase (PTK) activity in KB cells. In the presence of 1μg/ml of RC-160, a significant inhibition of total PTK activity was obtained (p<0.001), in KB cells (Fig. 22); the enzyme activity being reduced by 37%.

Fig. 22 shows the inhibition of PTK activity induced by long chain lipophilized derivatives of RC-160 such as myristoyl-RC-160, as compared to butanoyl-RC-160 and RC-160. The magnitude of inhibition in PTK activity, induced by lauroyl-RC-160, myristoyl-RC-160 or pamitoyl-RC-160 was found to be similar to RC-160, however the suppression of kinase activity was displayed at a 10-fold lower concentration, than RC-160 (p<0.01). However, butanoyl-RC-160 inhibits PTK activity at the same concentration as RC-160.

The treatment of KB cells in serum free medium with the cAMP analog dibutryl cAMP, resulted in a dose dependent increase in cell proliferation (Fig. 23A) with a maximal effect at 1μM (about 30% increase over control). EGF was found to potently stimulate intracellular cAMP levels in KB cells (Fig. 23B). The lipopeptides and RC-160 were found to potently inhibit EGF-stimulated intracellular cAMP concentrations (Fig. 24 and Table 9). Since, pertussis toxin alone inhibited EGF induced cAMP production in KB cells (Fig. 25), the effect of this toxin on the inhibitory action of RC-160 (and the lipopeptides) on intracellular cAMP levels, could not be evaluated.

**DISCUSSION:**

Oral cancer is a major health problem, not only in India but all over the world. Radiation therapy and surgery form the standard treatment strategy for oral cancers. However, surgical resection leads to severe physical disfigurement and dysfunction, leaving the patient with a very poor quality of life. Chemotherapy has a palliative role in the therapy of oral cancers but randomized prospective studies have failed to
Fig. 22  Inhibition of EGF-induced tyrosine kinase activity, by RC-160 and its lipophilized derivatives, in KB cells. The cells were treated with the above mentioned concentration of peptide for 20 minutes. The cells were lysed and the tyrosine kinase activity was determined using a broad specificity peptide substrate.
Fig. 23 Cyclic AMP causes proliferation in KB cells (A). Effect of EGF on intracellular cAMP levels (B), in KB cells.
Fig. 24 Effect of RC-160 and its lipophilized derivatives on EGF induced intracellular cAMP levels *in vitro*, in the human oral carcinoma cell line KB.
Table 9: Effect of lipopeptides and RC-160 on the inhibition of cAMP, in the human oral carcinoma cell line KB \textit{in vitro}. The optimum concentrations of the lipopeptides giving the maximum inhibition of cAMP is shown.

\begin{tabular}{l|l|ll}
\hline
SNo. & LIPOPEPTIDES & DOSAGE (\(\mu\text{g/ml}\)) & \% inhibition of cAMP \textit{in vitro} \\
\hline
1) & RC-160 & 1 & 65 \\
2) & Butanoyl-RC-160 & 1 & 61 \\
3) & Lauroyl-RC-160 & 1 & 60 \\
4) & Myristoyl-RC-160 & 1 & 66 \\
5) & Pamitoyl-RC-160 & 1 & 66 \\
\hline
\end{tabular}
Fig. 25 Effect of pertussis toxin on the inhibition of cAMP by RC-160 and its lipophilized derivatives, on the oral carcinoma cell line KB. KB cells were treated with 50ng/ml pertussis toxin, 18 hours prior to the assay, after which the cAMP determination was performed as described in "Materials & Methods".
demonstrate any conclusive benefit of chemotherapy (Kaur and Fee, 1993; Constenla et al., 1997).

The epidermal growth factor receptor (EGFR), observed to be highly overexpressed in squamous carcinomas is thought to be one of the biomarkers of malignant oral cancers (Partridge et al., 1988, Christensen, 1992). The overexpression of epidermal growth factor receptor (EGFR) in oral carcinomas, has led to extensive studies emphasizing the importance of EGF in the growth and neoplastic transformation of the oral epithelium (Christensen, 1998; Bergler et al., 1989). The somatostatin agonist, RC-160, has been found to be a potent inhibitor of EGF stimulated cell proliferation (Liebow et al., 1989), hence the anti-proliferative activity of RC-160 and its lipophilized derivatives was investigated in the human oral cell line KB. RC-160 was found to potently suppress EGF-induced proliferation of human oral carcinoma cell line KB in vitro.

The anti-proliferative activity of RC-160 seems to be mediated by somatostatin receptors on KB cells. The present study also demonstrates, for the first time the presence of somatostatin receptors on KB cells. Scatchard analysis demonstrates the presence of two distinct classes of receptors (high affinity and low affinity) on these cells. The K_d and B_max values, indicate that one class of receptors possesses high affinity and low capacity and vice versa. Moreover, RC-160 seems to interact with somatostatin receptors on oral carcinoma cells via a pertussis toxin insensitive GTP binding protein. These findings suggest that the somatostatin analog RC-160 has an attractive therapeutic potential, in the treatment of oral carcinoma.

The role of SSTR2 in the negative control of cell proliferation, by somatostatin analogs like RC-160 has been extensively studied (Reubi et al., 1994). As discussed previously, (page 48) the molecular interactions between RC-160 and SSTR2 are primarily hydrophobic in character. Hence, it may be conjectured that increasing the hydrophobicity of RC-160 should improve its receptor affinity and anti-proliferative activity. The receptor affinity (indicated by the IC_{50} values) of myristoyl-RC-160 does
not appear to be significantly different from RC-160. As seen in cell lines studied earlier, butanoyl-RC-160 has a lower affinity for somatostatin receptors on KB cells, as compared to RC-160.

The dose response of RC-160 (as well as its lipophilized derivatives), to inhibit cell growth in KB cells was found to be biphasic in nature, the maximal anti-proliferative activity being only manifested at an optimum peptide concentration. Butanoyl-RC-160 was found to be a less potent derivative of RC-160 than the other lipopeptides. It displays anti-proliferative activity at a 10-fold higher concentration as compared to RC-160. The anti-proliferative activity of RC-160 increases with lipophilization in KB cells, but saturates after octanoyl-RC-160, beyond which there is no increase in the anti-proliferative efficacy of lipopeptides. The lipopeptides manifest similar suppression in cell growth at a 10-fold lower concentration as compared to RC-160. Stearoyl-RC-160 was found to display lower anti-proliferative activity than pamitoyl- or myristoyl-RC-160, in EGF-induced proliferation of MIA-PaCa2 and DU145 cells. However, in human oral carcinoma cells stearoyl-RC-160 was equipotent to myristoyl- or pamitoyl-RC-160. Such differences in the relative efficacy of RC-160 and the lipopeptides can be attributed to tissue specific differences in the relative expression of somatostatin receptor subtypes on these cell lines.

The signaling pathways underlying the anti-proliferative activity of the lipopeptides appear to follow a similar pattern as RC-160. Thus, the stimulation of a protein phosphatase does not seem to be involved in the negative growth signal. The anti-proliferative activity of these peptides was not abrogated by sodium orthovanadate, a specific inhibitor of protein tyrosine phosphatase (PTPase) or okadaic acid, a specific inhibitor of serine threonine phosphatase 1A and 2A. Furthermore, the anti-proliferative activity of RC-160 and the lipopeptides is insensitive to pertussis toxin, indicating that the anti-proliferative signal is independent of pertussis toxin sensitive GTP binding proteins.
The down regulation of PTK activity, has been an extensively investigated therapeutic strategy to suppress cell growth in human cancers (Volberg et al., 1997). Studies by Pawlikowski et al. (1998), and Keri et al. (1996) have implicated the inhibition of tyrosine kinase activity, as a possible mechanism underlying the antineoplastic activity of somatostatin analogs. RC-160 and the lipopeptides were found to potently inhibit EGF stimulated PTK activity. The long chain lipopeptides like myristoyl-RC-160 displayed a similar inhibition of tyrosine kinase activity at a 10-fold lower concentration, compared to butanoyl-RC-160 and RC-160 itself. Pawlikowski et al. (1998) have speculated that SSTR1 mediates the suppression of PTK activity in murine colonic tumors, by stimulating PTPase activity. Controversy exists regarding the coupling of SSTR1 to PTPase activity in cells. Data from some workers show that somatostatin induced no stimulation (Reardon et al., 1997), very low stimulation (Buscail et al., 1995), or efficient stimulation of phosphatase activity (Florio et al., 1994) in CHO cells expressing SSTR1 or SSTR5. Florio et al. (1994), have measured PTPase activity after 2 hour stimulations with somatostatin, whereas Reardon et al. (1997) and Buscail et al. (1995) have principally used 10-15 minutes stimulation. In this study, the measurement of tyrosine kinase activity was done after a 15 minute stimulation by RC-160 (or the lipopeptides), similar to the protocol of Reardon et al. (1997) and Buscail et al. (1995). The somatostatin receptor subtype 5 is known to mediate its biological activities through a PTPase independent pathway. The somatostatin receptor SSTR1 has also been shown to mediate its biological activities by the activation of the MAP kinase cascade (Florio et al., 1999). In accordance, our data seems to suggest that the receptor subtypes SSTR1 and/or SSTR5 are possibly overexpressed on KB, since the anti-proliferative activity is not mediated by the stimulation of PTPase activity, or serine/threonine phosphatase 1A or 2A activity. It is also possible that the phosphorylation-dephosphorylation phenomena studied by us is different from the one studied by Florio et al. (1994) or Buscail et al. (1995). Major voids still remain in our understanding of somatostatin receptor subtype-specific molecular signals responsible for the activation of various phosphatases. Much of our current knowledge is based on CHO cells transfected with somatostatin receptor subtypes, and should be interpreted with caution, given
the limitations of these systems. The signaling pathways, or more specifically the phosphorylation-dephosphorylation phenomena (in response to these peptides) can vary from cell line to cell line, depending on the relative number and density of somatostatin receptor subtypes expressed on the cell surface (Weckbecker et al., 1993).

The second messenger cAMP has been known to stimulate proliferation in certain mammalian cell lines, and has also been found to act as a mediator of mitogenic hormones (Dumont et al., 1989). However, cAMP has been also found to inhibit cell proliferation (Tortora et al., 1991) in certain mammalian cells. Due to this dual behavior of cAMP on cell proliferation, the suppression of intracellular cAMP cannot be always correlated to the anti-proliferative activity of somatostatin. The cAMP analog, dibutryl cAMP stimulated the growth of KB cells in a dose dependent fashion, suggesting that the suppression of intracellular cAMP was one of the signaling pathways mediating the anti-proliferative activity of these lipopeptides.

RC-160 and the lipopeptides potently inhibited intracellular cAMP formation in KB cells. All the peptides inhibit cAMP to the same magnitude. Hence, the inhibition of cAMP cannot solely explain the improved anti-proliferative activity of the lipopeptides. The anti-neoplastic activity of bioactive peptides, may not be the consequence of a single signaling pathway (like the inhibition of cAMP); most often it is the result of a combination of multiple interactive signaling cascades, out of which some (like inhibition of PTK activity) may play a more prominent role than the others. Somatostatin analogs are coupled to adenylyl cyclase in intracellular signal transduction (Reisine et al., 1995; Carruthers et al., 1999). Somatostatin analogs do not directly affect activity of adenylyl cyclase, but they inhibit the activation of adenylyl cyclase through a possible interaction through a G-protein (Qin et al., 1995).

The results obtained in this study, suggests a therapeutic potential for the somatostatin analog RC-160, in the treatment of oral carcinoma. Lipophiziation of
RC-160 with fatty acids appears to enhance its anti-proliferative activity in human oral carcinoma, without altering the signaling pathways underlying its anti-neoplastic activity.