To Meaghan McPhee

CHAPTER 1: INTRODUCTION

1.1 Preface

This thesis will be discussing stabilized combi-molecules for the treatment in breast cancer. Due to the fact that these molecules novel drugs for use in breast cancer, the first section of the introduction will summarize the current method of treatment available for breast cancer. As well, a brief description of the recent advances made in the field of breast cancer chemotherapeutics will be provided. The next two sections of the introduction will focus in EFGR and current target of EGFR and DNA damaging agents because these molecules combine both EGFR inhibitory and DNA damaging capabilities. The final section will give an overview of the combi-targeting concept established in our lab as well as discuss the theory behind the synthesis of the molecules presented herein.

1.2 Breast Cancer: Introduction and Current Treatment

Breast cancer is the most common type of cancer among Canadian women. In 2008, it is projected that approximately 22 400 women will be diagnosed with cancer and nearly 5 300 women will die from the disease (Canadian Cancer Society). These statistics are evidence that research towards a cure for breast cancer is very important. The present treatment regimen begins with diagnostic imaging for detection and screening. If possible, the patient can undergo surgical resection of the primary tumour followed by removal and pathological evaluation of the ipsilateral axillary nodal basin. Some patients then receive radiation therapy to reduce or prevent local recurrence. Finally, patients may receive systemic therapy as a replacement for or in addition to radiation therapy to decrease the risk of local recurrence and distant metastasis (Moulder and Hortobagyi, 2008). This review will explore the advances and potential directions in this final step.

Systemic therapy can be used for both neoadjuvant and adjuvant therapy. Although more commonly used in adjuvant therapy, use of systemic therapy in neoadjuvant therapy also has its advantages. First, patients who have inoperable tumours may experience tumour shrinkage and become eligible for tumour resection. Shrinkage of an already operable tumour may also reduce the extensiveness of the resection surgery. Second, the drug's effects on the tumour can be monitored and modifications can be made to the treatment program. Finally, tumour tissue can be observed before and after treatment in order to determine the correlation between the response of the tumour and its molecular parameters (Moulder and Hortobagyi, 2008).

The first class of systemic therapy is comprised of hormone therapy drugs that target the estrogen receptor (ER). One type of hormone therapy drug is the selective estrogen response modulators (SERMs). Two examples of SERMs are Tamoxifen and Raloxifene. Both of these molecules, as well as other SERMs, have a structure similar to estrogen. However, once bound to ER, Tamoxifen and Raloxifene elicit different responses than estrogen. Both Tamoxifen and Raloxifene are currently used to treat patients that have ER-positive breast cancer.

A second class of hormone therapy drug is the third-generation aromatase inhibitors (AIs). AIs have been found to have greater efficacy than SERMs in postmenopausal women with ER-positive breast cancer. The list of AIs currently being used in the clinic includes Anastrozole, Letrozole, and Exemetane (Moulder and Hortobagyi, 2008).

A third type of systemic therapy is chemotherapeutics. The main type of chemotherapy agents is the anthracyclines, which include doxorubicin and epirubicin. Anthracyclines inhibit topoisomerase II, which is required for maintaining DNA structure during replication, transcription, recombination, and chromosomal condensation and decondensation, all of which are necessary for cell survival. Another type of chemotherapy agent is the taxanes. Taxanes bind

β-tubulin, stabilize microtubule formation, and arrest mitosis. Paclitaxel and docetaxel are two examples of taxanes that are currently being used for breast cancer therapy. Two additional breast cancer chemotherapeutics are Vinorelbine, a vinca alkaloid, and Gemcitbine, a deoxycytindine analog (Moudler and Hortobagyi, 2008). A list of some of the available breast cancer chemotherapy agents can be found in Table X.

Recently, advances have been made to the field of chemotherapy. With the discovery of the importance of the receptor tyrosine kinases (RTKs) in cancer signalling, new therapeutics have been created to target the signalling pathways activated by RTKs such as the vascular epidermal growth factor receptor (VEGFR) and the erbB family of receptors. So far, the primary advancement has been made in therapeutics that target erbB2 (HER2) of the erbB family of receptors. However, one recombinant, humanized, anti-VEGF antibody called Bevacizumab has been discovered (Moulder and Hortobagyi, 2008).

The HER2 receptor is overexpressed in approximately 20-30% of breast cancer cases and is correlated with a poor prognosis and decreased survival. Thus far, the most celebrated HER2 targeting therapeutic is Trastuzumab (Herceptin). Trastuzumab is a recombinant, humanized monoclonal antibody that sequesters HER2 and prevents it from binding with other erbB receptors. It has been shown that Trastuzumab treatment inhibits cell proliferation and induces apoptosis in cancer tumour cells (Moulder and Hortobagyi, 2008). Despite the increased expression of EGFR in breast cancer cells, a single agent that targets only EGFR is not yet available for clinical use (Moulder and Hortobagyi, 2008).

Table. A table containing a few of the available chemotherapeutics for use in breast cancer therapy (Moulder and Hortobagyi, 2008).

Table 1 Chemotherapy agents with single-agent activity

Drug	Response rate (%)
Doxorubicin	10–50%
Epirubicin	13–48%
Liposomal doxorubicin	10–50%
Docetaxel	q. 21 days: 18–68% q. 7 days: 33–50%
Paclitaxel	q. 21 days: 16–62% q. 7 days: 22–53%
ABI-007 (Abraxane)	33–48%
Capecitabine	20–35%
Vinorelbine	25–50%
Gemcitabine	12–37%
Cisplatin/carboplatin	9–51%
Irinotecan	14–23%

1.3. ErbB Receptors: Introduction and Downstream Signalling Pathways

1.3.1. ErbB receptor family

The erbB family of tyrosine kinase receptors consists of four members: erbB1 (EGFR, HER1), erbB2 (neu, HER2), erbB3(HER3), and erbB4(HER4). These receptors play crucial roles in cellular functions such as development, proliferation, differentiation, migration, survival, and metabolism (Olayioye, et al.,2000; Stamos *et al.*, 2002; Zhang *et al.*, 2006). The expression of the four erbB receptors is diffuse throughout the human body and can be found in epithelial, mesenchymal, and neural tissues. Their many functions result from their ability to associate with multiple ligands ~~as well as~~ and to form both homo- and heterodimers, which each activate specific downstream signalling pathways (Olayioye, *et al.*, 2000).

One of the most pivotal discoveries leading to the advent of the erbB family of receptors occurred in 1962 when Cohen *et al.* discovered a new bioresponse-mediating protein. This protein was named the epidermal growth factor (EGF) because it could activate proliferation of epidermal cells (Cohen *et al.*, 1962). Thirteen years later, Carpenter *et al.* identified a target of EGF on the cell surface using ^{125}I-labeled EGF. Soon after, this unknown target of EGF was further characterized using A-431 epidermoid carcinoma cells as a 170kDa membrane protein, which incorporated increased concentrations of ^{32}P upon association with EGF (Carpenter *et al.*, 1975). Due to its association with EGF, the protein was named epidermal growth factor receptor (EGFR). The tyrosine kinase activity of EGFR was discovered in 1980 (Ushiro *et al.*, 1980). It was not until 1984 that the other three members of the erbB family were discovered using a cDNA library screening (Gschwind *et al.*, 2004). Each of the subsequent members of the erbB family had very similar sequences and were named human EGFR-related (HER) 2, 3, and 4 (Gschwind *et al.*, 2004).

→ No should be a 1984 Ref.)

1.3.2. ErbB receptors: structure

The erbB family of receptors are all single-chain molecular glycoproteins and, although each receptor is distinct, they share the same general structure. First, each receptor has an extracellular domain made up of approximately 620 amino acid residues. The extracellular domain contains the important ligand binding domain. Although erbB2 has no known ligands, the general structure of the extracellular domain is conserved. The extracellular component of the receptor is further divided into four smaller domains known as L1, CR1, L2, and CR2 (Burgess *et al.*, 2003). L1 and L2 are homologous domains that are understood to be part of the leucine rich repeat family (Ward *et al.*, 2007). CR1 and CR2 are cysteine rich domains that contain multiple small disulphide bonded modules. Domain CR1 has 8 disulphide bonded molecules in the configuration C2C2C2C1C1C1C1C1. In this configuration, C2 represents two intertwined disulphides that connect side chains in the arrangements Cys1-Cys3 and Cys2-Cys4, while C1 represents modules defined by a single disulfide bond. CR2 has only seven disulfide bonds in the order C2C1C1C2C1C1C2. Crystalography studies have determined that the CR domains form laminin-like folds and the L domains form a right-handed β-helix (also known as a solenoid). More specifically, the L domains form a six-turn right-handed β-helix that is capped at each end by an α–helix and a disulphide bond. The L2 domain forms the primary docking site for the ligand molecules, with L1 playing a supporting role in binding of the ligand (Burgess et al., 2003).

The second domain of the erbB receptor is the single-pass transmembrane helix, which contains approximately 23 amino acid residues (Hubbard, 2006; Burgess *et al.*, 2003). The next main component of the receptor is the intracellular domain, which is connected to the transmembrane domain by small juxtamembrane region. The intracellular domain contains the

tyrosine kinase activity in all of the erbB receptors except for one. ErbB3 has an inactive kinase domain. Finally, each membrane has an essential carboxyl-terminal (C-terminal) tail segment containing important phosphorylation sites that are necessary for the activation of the receptor's kinase activity and the binding of downstream mediators (Stamos *et al.*, 2002; Zhang *et al.*, 2006). A simple representation of the structure of the receptors can be seen in Figure 1.

1.3.3. ErbB receptors: ligands

The high degree of the erbB receptors' signalling diversity is due, in part, to their many ligands. Like other RTKs, the kinase activity of the erbB receptors is greatly increased upon ligand binding. All the receptors expect erbB2 have many ligands, each with differing affinities and effects on the receptor. The binding of a ligand can cause the bound receptor to preferentially dimerize with another receptor and activate a downstream pathway, resulting in a multitude of ligand-specific cellular functions. The erbB family of receptors have many ligands, such as epidermal growth factor (EGF), amphiregulin (AR), transforming growth factor alpha (TGFα), betacellulin, heparin-binding epidermal growth factor (HB-EGF), epiregulin (EPR), biregulin (BiR), and neuregulin 1-4 (NRG1-4) (Olayioye et al, 2000, Jones, et al., 1999, Harris et al, 2003). The receptors with which each ligand can associate can be seen in Table 1. Some ligands can interact with more than one receptor, yet not all of the interactions are primary interactions. For example, neuregulin-2 can bind to both erbB3 and erbB4, but preferentially binds to erbB4. The interaction between the receptor and the ligand plays an important functional and regulatory role. The binding affinity of each ligand affects both the strength and duration of the interaction. For example, a ligand that binds with a low affinity can prevent the downregulation of a signal and the degradation of the receptor by sequestering it. pH also plays

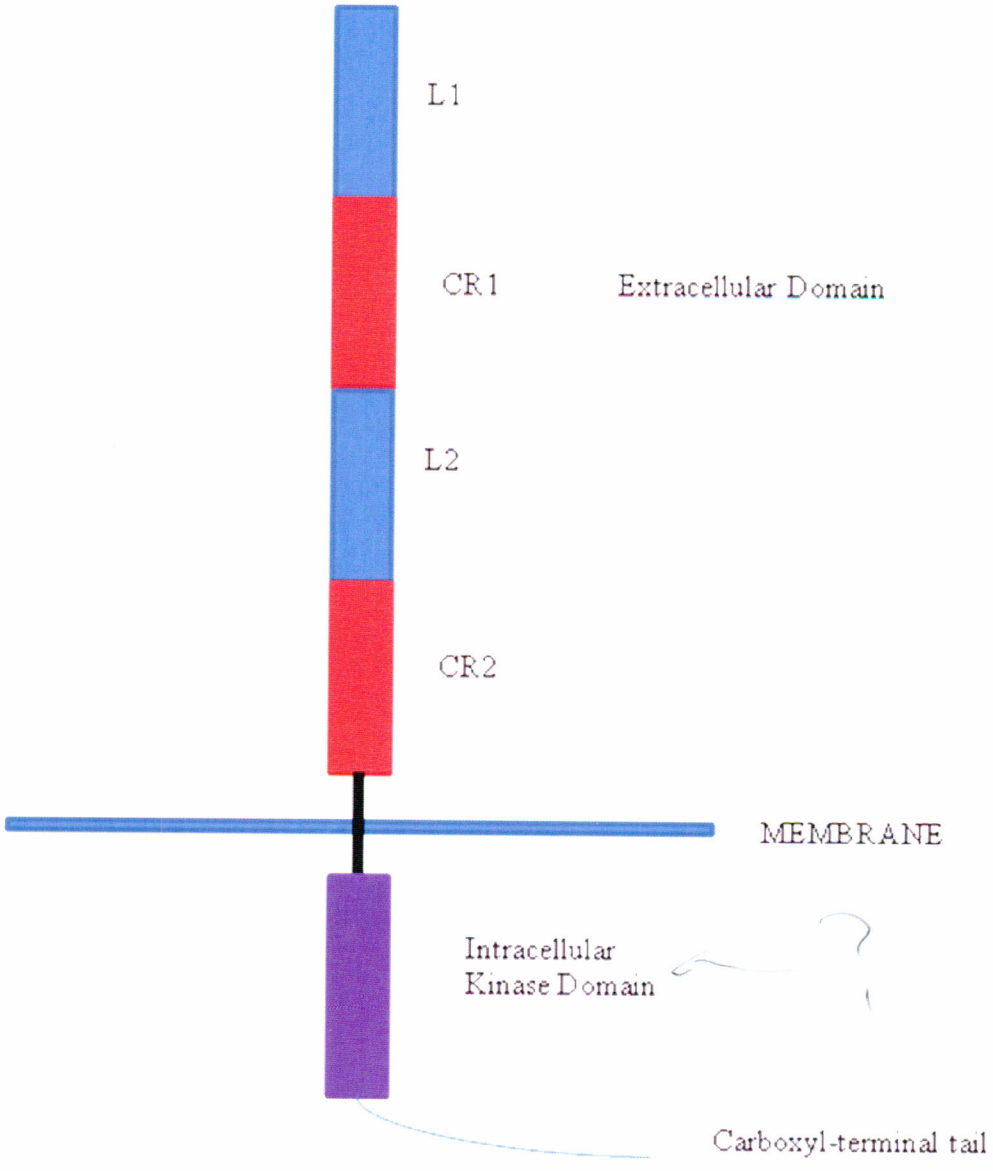


Figure. Simple representation of the structure of erbB family of receptors.

Table 1. The erbB receptors and their repsective ligands (Olayioye *et al.*, 2000, Jones *et al.*, 1999).

erbB1	**erbB2**	**erbB3**	**erbB4**
EGF		NRG1	NRG1-4
AR		NRG2	BTC
TGFα			HB-EGF
BTC			EPR
HB-EGF			
EPR			

an important role in the downstream signalling of the erbB receptors. A ligand that can tolerate low pH, such as EGF, can target receptors to the lysosome, whereas a ligand that dissociates from its receptor at endosomal pH results in the recycling of the receptor. Neuregulin-1 (NRG-1) and transforming growth factor alpha (TGFα) are both ligands that cause their respective receptors to be recycled (Olayioye *et al.,* 2000).

1.3.4. ErbB receptors: ligand binding

Binding of the ligand to the receptor is the critical step in erbB receptor tyrosine kinase activation. The ligand molecule binds to both L1 and L2 in the extracellular domain of one receptor (Ward et al., 2007). When the ligand binds, the intramolecular forces between CR1 and CR2 are broken and the CR1 domain is exposed. The CR1 domain facilitates the dimerization of the erbB receptors and is called the "dimerization arm" (Dawson et al., 2007). Although the ligands play a crucial role in the dimerization of the erbB receptors, they do not bridge the two receptors. Rather, ligands bind to receptors in a 1:1 ratio. In other words, the ligand of one receptor in the dimerization pair does not interact with the other receptor.

1.3.5. ErbB receptors dimerization

The erbB receptors have two conformations. When the receptor is not bound by a ligand, it is in the tethered, or autoinhibited, conformation (Figure 2 a). Upon binding of the ligand, the receptor undergoes conformational changes to adopt the extended conformation (Figure 2 b). In the extended conformation, the dimerization arm is extended and is capable of binding to another erbB receptor, forming a homo- or heterodimer. Additionally, the dimerization loop of the CR1

domain becomes exposed and can interact with another erbB receptor (Figure 2 c) (Ferguson, 2004). The dimerization loop of the four erbB receptors is highly conserved, which possibly

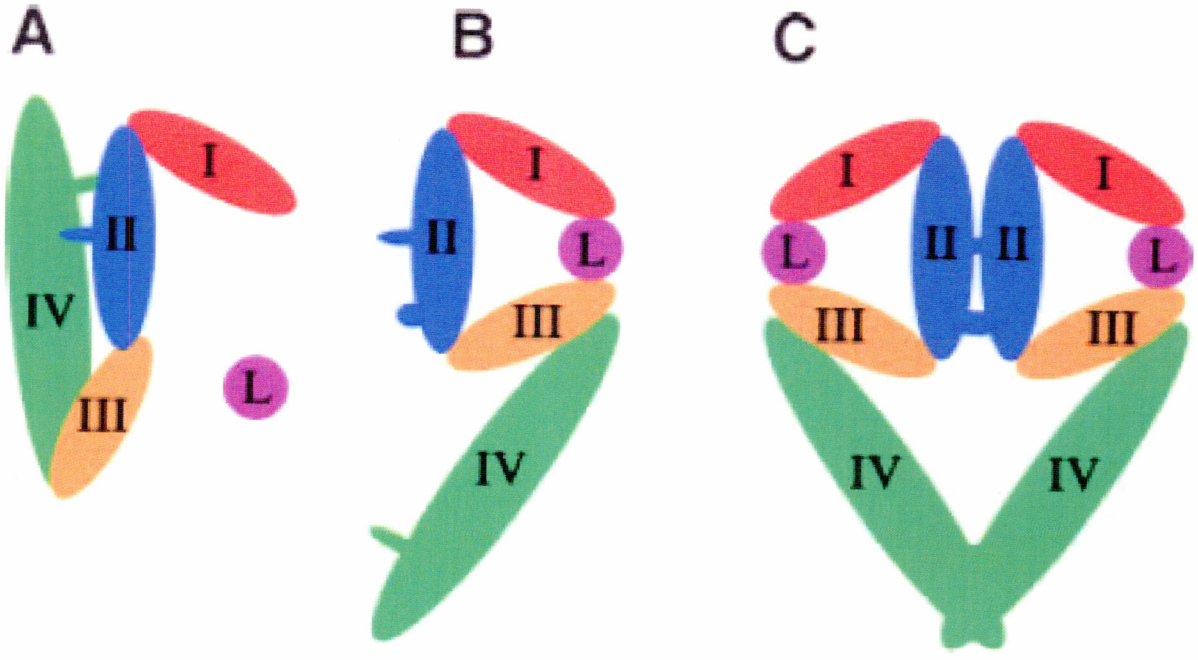


Figure 2. A) erbB receptor in the inactive or tethered conformation. B) erbB receptor with a ligand bound at domains I and III. C) Representation of two erbB receptors forming a homo- or heterodimer (Riese II *et al.*, 2007). Note L represents ligand

explains the observation that erbB receptors not only form homodimers, but also heterodimers with other members of the erbB family. Interestingly, erbB2 is the preferred heterodimerization

partner for all of the erbB receptors. This is due to the fact that erbB2, which has no known ligands, is constitutively in the extended conformation. (Schlessinger, 2002).

Upon dimerization, specific sites in the C-terminal become phosphorylated. Once phosphorylated, they become binding sites for downstream signalling molecules that contain SH2 or PTB domains (Zhang *et al.,* 2006).

1.3.6. Ras/Raf/MEK/ERK pathway

One of the two main downstream pathways of EGFR is the Ras/Raf/MEK/ERK pathway (Jimeno and Hidalgo, 2006). This pathway plays a role in promoting both cell proliferation and cell survival (Roberts and Der, 2007). The first protein in this pathway is Ras, a GTP-binding protein that exerts its action at the cell membrane. Translocation of the Ras protein to the cell membrane requires either farsenylation by farnesyl transferase or geranylgeranylation by geranylgeranyl transferase on a cystiene residue (McCubrey, *et al.*, 2006). Upon activation of EGFR by EGF, the Shc/Grb2/SOS complex is activated. Son of sevenless (SOS) is transported to the membrane by growth-factor-receptor-bound protein 2 (Grb2) due to its ability to recognize tryrosine phosphate docking proteins in the C-terminal of EGFR (Kolch, 2000; Roberts and Der, 2007). Activation of this complex results in the conversion of Ras-GDP to Ras-GTP causing a change in conformation and activation of Ras (Zhao *et al.*, 2007).

It is the conversion of Ras-GDP to Ras-GTP that recruits the next protein in the Ras/Raf/MEK/ERK pathway, Raf, to the cell membrane. Raf is a serine/threonine (S/T) kinase. Three isoforms of Raf have been identified: A-Raf, B-Raf, and Raf-1 (McCulbrey *et al.*, 2006). All three isoforms contain the CR1, CR2, and CR3 domains. CR1 is the Ras-binding domain and

CR3 is the kinase domain containing the phosphorylation sites necessary for Raf activation (Chang *et al.*, 2003).

Activation of Raf requires many steps. First, Raf must be translocated to the cell membrane by Ras. Following localization at the membrane, two Raf proteins form a dimer. Next, phosphorylation and dephosphorylation at many different amino acid residues occur. In its inactive form, Raf is phosphorylated at S43, S259, and S621. Dephosphorylation of S621 by an unknown phosphatase allows dephosphorylation of S259 by protein phosphatise 2A (PP2A). Dephosphorylation of S259 leads to disassociation of Raf from the inhibitory protein 14-3-3. Once released by 14-3-3, Raf becomes phosphorylated at S338, tyrosine(Y)340, and Y341. It believed that Src is responsible for the phosphorylation of Raf at Y340 and Y341. This final step completes the activation of Raf (McCulbrey *et al.*, 2006).

Once activated, Raf phosphorylates MAPK/ERK kinase (MEK) on two serine residues in the catalytic domain transforming MEK from its inactive to active state. MEK is a dual specificity kinase for Y and S/T (McCulbrey *et al.*, 2006).

MEK phosphorylates the final kinase in the pathway, which is extracellular-signal-regulated kinase 1,2 (ERK1,2). MEK activation of ERK1,2 occurs through the phosphorylation of a Thr-Glu-Thr sequence in activating motif of the ERK1,2 protein. Activated ERK1,2 regulates many downstream targets (Kolch, 2000; McCulbrey *et al.* 2006). As many as 160 targets of ERK have been identified. Although most of the targets of ERK are located in the nucleus, some are found in the cytoplasm and other organelles (Roberts and Der, 2007). Upon activation, ERK is translocated to the nucleus where it phosphorylates many transcription factors, such as Ets-1, AP-1, c-Jun, and c-Myc. (Chang *et al.*, 2003; Roberts and Der, 2007;

McCulbrey *et al.*, 2006). These transcription factors are important for both transcription and cellular proliferation.

1.3.7. PI3K/Akt pathway

The second main downstream pathway of the EGFR is the PI3K/Akt pathway (Akt pathway). The Akt pathway has a role in many cellular processes, including nutrient metabolism, smooth muscle stimulation, angiogenesis, cell growth, proliferation, and apoptosis. The Akt protein is a serine/threonine protein kinase and is sometimes referred to as protein kinase B (PKB). Akt requires phosphorylation of both threonine 308 (T308) and serine 473 (S473) for its activation. The structure of Akt consists of an amino terminal pleckstrin homology (PH) domain, a central kinase domain, and a cerboxy-terminal regulatory domain (Bianco *et al.,* 2006; Wang *et al.*, 2006).

The EGFR indirectly activates Akt through phosphoinositide 3-OH kinase (PI3K). Upon activation of the EGFR, the p85 subunit of PI3K is recruited to the receptor and binds to the carboxy-terminal tail via its SH-domain. Once bound, p85 recruits the catalytic subunit of PI3K forming the activated PI3K. In turn, PI3K generates phosphatidyl inositol (3,4,5)-tris-phophates (PIP3) in the plasma membrane, which affects the location and conformation of Akt. Finally, Akt activation is completed by phosphorylation of two 3-phoshpoinositide dependent protein kinases (PDKs), specifically, PDK1 phosphorylation of T308 and PDK2 phosphorylation of S473 (Velling *et al.*, 2008).

The Akt pathway is best known for its role in cell survival through inhibition of apoptosis. There are two mechanisms by which Akt prevents apoptosis. First, it inhibits the factors necessary for transcription of proteins that regulate apoptosis, such as the forkhead

transcription factors. Secondly, Akt phosphorylates and inhibits positive mediators of apoptosis, such as caspase 9, BAD, NF-KB, and glycogen synthase kinase 3 β (Okano *et al*, 2000).

As was mentioned above, Akt also plays a role in the regulation of cell growth and proliferation. One way Akt affects proliferation is through indirect, positive regulation of a key regulator of cell growth and proliferation, mammalian target of rapamycin (mTOR). Specifically, Akt inhibits negative regulators of mTOR, such as tuberin (TSC2) (Bianco *et al*, 2006).

1.4. Targeting EGFR

Since the discovery that many growth factor receptors are overexpressed in cancers, novel therapeutics have sought to inhibit these receptors and their downstream signalling pathways. In particular, it was determined that EGFR is overexpressed in many cancers, such as breast, ovary, lung, and head and neck, to name a few (Traxler *et al.*, 2001). The high degree of overexpression has made EGFR a popular target for cancer treatment. Two methods for inhibiting EGFR are currently being explored and a brief summary of the current successes in each area is provided below.

1.4.1. Monoclonal Antibodies

The first strategy for targeting EGFR is the use of monoclonal antibodies that bind to the ligand binding domain, while preventing the ligands from associating with their receptor and activating downstream signalling. The only anti-EGFR antibody that has been approved thus far is cetuximab (Erbitux), a chimeric monoclonal G1 (IgG1). In 2004, Cetuximab was approved by the United States food and Drug Administration (FDA) for the treatment of colorectal cancer (Schiller, 2008). It is now being used to treat both metastatic colorectal cancer and squamous cell

carcinoma of the head and neck. Cetuximab can inhibit cell growth and proliferation as well as cause the internalization and degradation of the EGFR. Currently, cetuximab is still in clinical trials for other types of cancers including pancreatic, breast, and cervical (Rocha-Lima *et al.*, 2007).

Although only one anti-EGFR antibody is available for treatment, many others are in different phases of clinical trials. One such antibody that is currently in clinical trials is matuzumab, a humanized IgG1 that contains only 5% murine content. Phase I clinical trials have indicated that Matuzumab is a potential therapeutic for non-small cell lung cancer (NSCLC), gastroesphageal, colorectal cancers, and possibly other solid tumours. As of 2008, matuzumab was in large scale clinical trials to assess efficacy in a wide scope of cancers (Schiller, 2008).

Another antibody currently in clinical trials is panitumumab. Panitumumab is a novel antibody because it is the first fully humanized antibody and does not contain any murine components. A fully humanized antibody is advantageous because murine components can elicit adverse immune responses in patients. As of 2007, panitumumab was in phase III clinical studies to assess its efficacy in a randomized trial (Carteni *et al.*, 2007).

1.4.2. Small Molecule Tyrosine Kinase Inhibitors

The second method of targeting EGFR is small molecule tyrosine kinase inhibitors (TKIs). TKIs bind to the adenosine triphosphate (ATP) binding site in the kinase domain of the receptors, prevent ATP from binding, and consequently, inhibit autophosphorylation of the receptor. The first TKI to be approved by the FDA in the United States was gefitinib (Iressa® AstraZeneca) in 2003. However, after further trials failed to increase survival in NSCLC patients, geritinib was removed from clinics except for patients that were already benefiting from

the drug, had benefited from the drug in the past, or were actively participating in clinical trials (Marshall, 2006).

The second TKI currently being used in the clinic is erlotinib (Tarceva® OSI Pharmaceuticals in collaboration with Genentech and Roche Pharmaceuticals). Like other TKIs, erlotinib is administered orally. It was approved by the FDA in 2004 for treatment in locally advanced or metastatic NSCLC and in 2005 for use in locally advanced, unresectable, or metastatic pancreatic cancer in combination with the drug gemcitabine. A key advantage of erlotinib is its selectivity for EGFR and its ability to inhibit the tyrosine kinase activity of the mutant form of EGFR, EGFRvIII (Marshall, 2006).

The most recent addition to FDA approved EGFR TKIs is lapatinib (GW572016 GlaxoSmithKline). Lapatinib is a dual tyrosine kinase inhibitor that can reversibly inhibit both EGFR and erbB2 (Jimeno and Hidalgo, 2006).

1.5. DNA Damage: Triazenes

Cytotoxic agents are commonly used in cancer therapeutic and exert their action by damaging DNA and killing cells. One class of cytotoxic agents is triazenes. Two triazenes currently used in the clinic are temozolomide (TMZ) and dacarbazine (DTIC). Both TMZ and DTIC are alkylating agents. Upon nucleophilic attack by water, they release their active moiety which is the triazenyl group. The triazenyl group contains three adjacent nitrogen atoms that are responsible for the cytotoxic activity of triazenes. In general, trizenes release an alkyldiazonium cation, which contains the triazenyl group. TMZ and DTIC both release methyldiazonium ions. (Figure____) Once released, the methyldiazonium cation reacts with purinic bases, particularly guanine. As a result of the reaction between the cation and the base, a methylated base is produced.

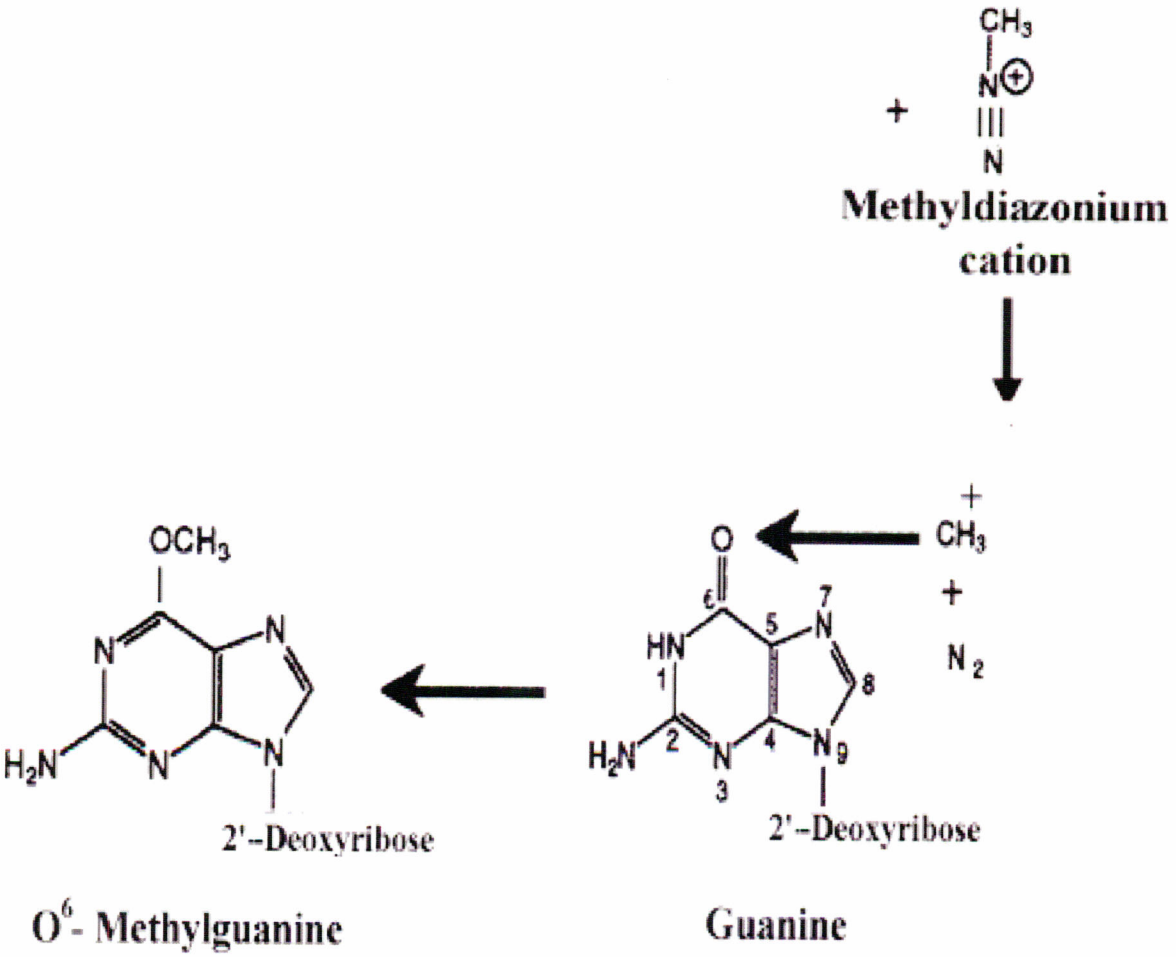


Figure. Formation of O6-methylguanine as a result of the interation between a methyldiazonium ion and guanine (Marchesi *et al.*, 2007).

Although N^7-methylguanine is the main adduct created, O^6-methylguanine (Figure ___) is responsible for the cytotoxic effects. The addition of the methyl-group at the O6-position prevents proper hydrogen bonding between guanine and cysteine causing the O^6-methylguanine to preferentially pair with thymine (Figure ____). If the mismatch is not repaired, the synthesis of DNA, RNA, and proteins is inhibited and the cell dies. It is the ability of triazenes to kill cells that makes them effective cancer therapeutics (Marchesi *et al*, 2007).

1.6. Combi-Targeting Concept

1.6.1. Background

Many attempts to treat cancer with single therapeutics have failed due to the ability of cells to bypass blockades in their signalling cascades. As a result, the most common treatment of cancer involves the combination of two or more drugs. Drug cocktails, as they are commonly called, have increased toxicities with their increased efficacy. Our lab has developed a novel concept that hope to achieve the positive effects of combination therapy while reducing the toxicity. In one approach, we have combined a quinazoline moiety that can inhibit the tyrosine kinase (TK) activity of EGFR with a triazene chain that damages DNA (Matheson *et al.*, 2004). A simple schematic of this class of molecules, known as combi-molecules, can be seen in Figure X. The initial combi-molecule TZ-I can directly bind to and inhibit the TK activity of EGFR. The molecule can further generate another inhibitor of EGFR, I, and a DNA damaging triazene, TZ (Brahmi, F. *et al.,* 2002). The goal is to down-regulate the anti-apoptotic pathway by inhibiting the TK activity of EGFR in order to increase the cytotoxic effect of the DNA damaging moiety. As well, through combining the DNA damaging agent with an inhibitor of

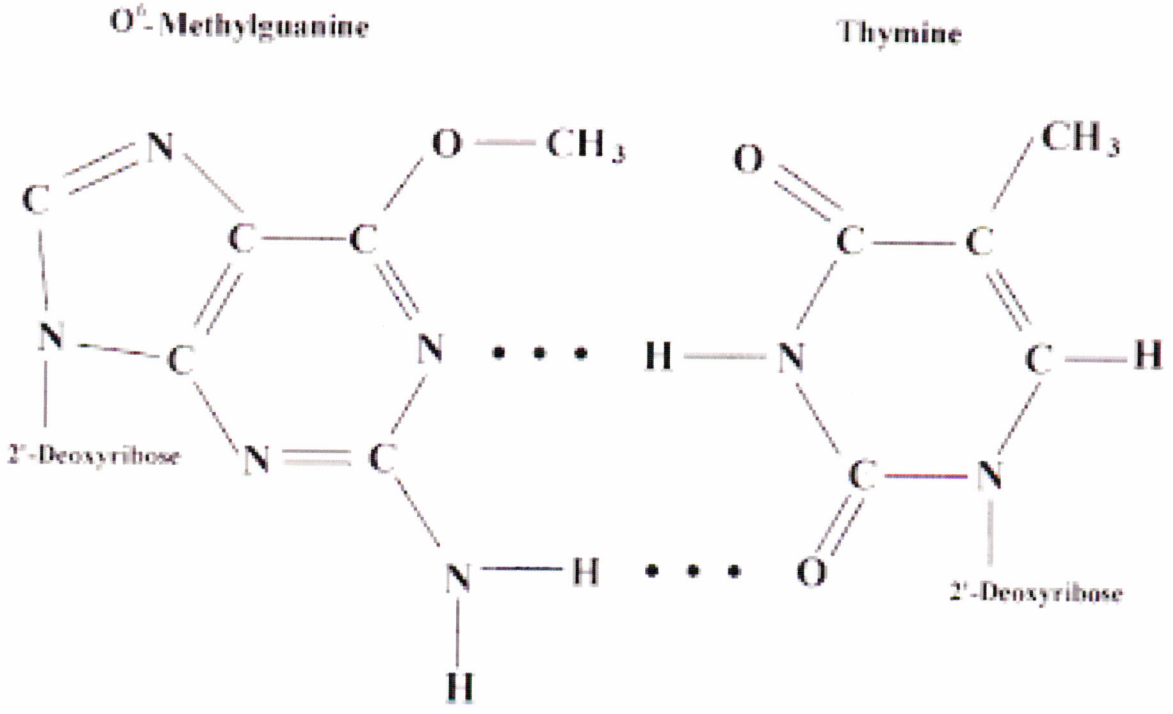


Figure. Preferential binding of O6-methylguanine with thymine (Marchesi *et al.*, 2007).

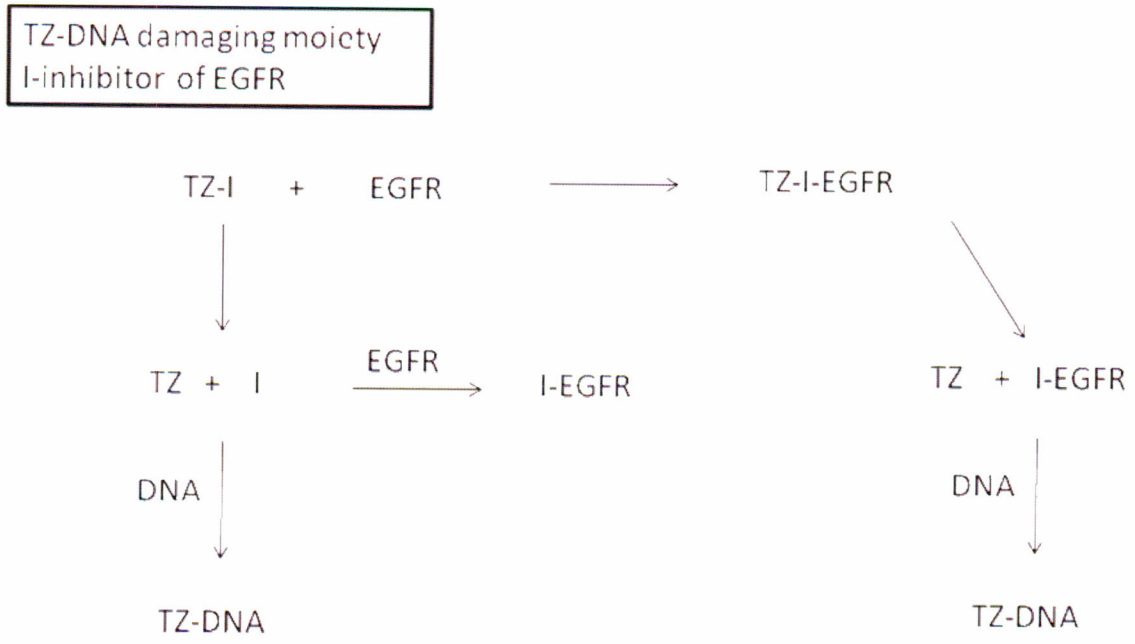


Figure. A simple representation of the combi-targeting concept.

EGFR we hope to decrease the toxicity by targeting the cytotoxic activity to ~~cells the~~ cancer cells that overexpress EGFR.

1.6.2. Past success of combi-molecules

The feasibility of combi-molecules that combine an inhibitor of EGFR and a DNA damaging moiety was been confirmed, in 2001, by the creation of SMA41 (Figure___) (Matheson *et al.*, 2004). SMA41 was shown to cause DNA damage, block EGFR autophosphorylation, and have antiproliferative effects *in vivo* (Matheson *et al.*, 2001; Matheson *et al.*, 2004). Although SMA41 molecule proved the efficacy of our ~~combi-molecules~~ that combine an EGFR-TK inhibitor and a DNA damaging agent, the EGFR-TK inhibitory potency was moderate and it had poor water solubility. To circumvent these problems, the molecules RB24 and RB107 were created (Figure X). In an attempt to increase the EGFR-TK inhibitory potential of the molecules, a methyl group was replaced with a less bulky appendage, such as a chloro-group (RB107) or bromo-group (RB24), in order to allow better binding to the ATP binding pocket on EGFR. As well, an acetoxymethyl-group was added to the N3 position of the triazene chain to increase water solubility (Banerjee *et al.*, 2003). Both RB24 and RB107 were shown to have good affinity for the EGFR and significant antiproliferative efficacy in DU145 cells. RB107 was also shown to significantly block the autophosphorylation of the EGFR and cause more DNA damage than Temodal® (TEM) (also assessed in DU145 prostate cancer cells). Due to the superior water solubility of RB107compared to RB24, it was choosen for *in vivo* studies and proved to have good efficacy. (Results not published) However, the short half-life of RB107 meant that it was not suitable for further *in vivo* kinetic studies. This final result led to the creation of the series of molecules that are the subject of this thesis.

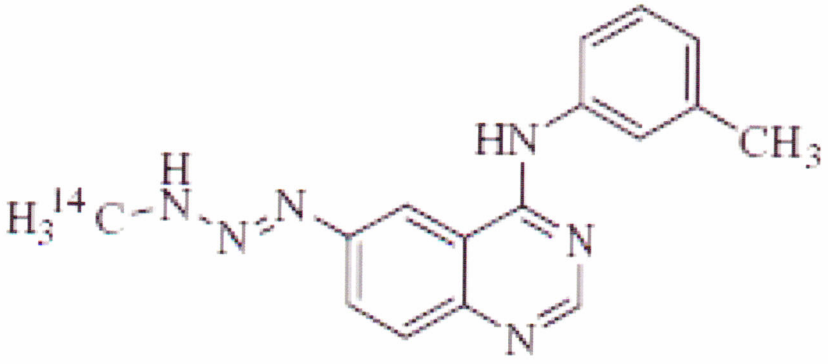


Figure. Structure of SMA41 (Matheson, *et al.*, 2003).

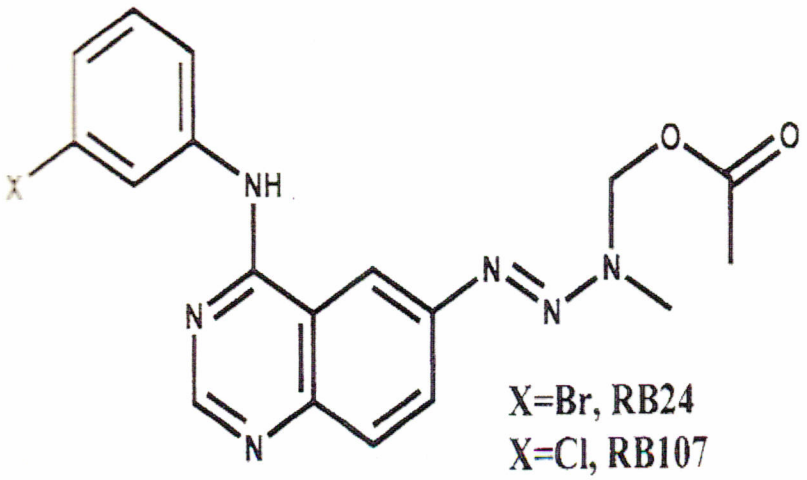
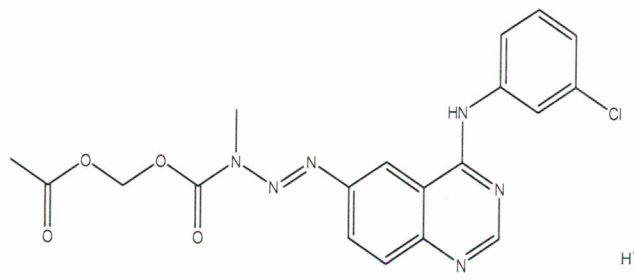


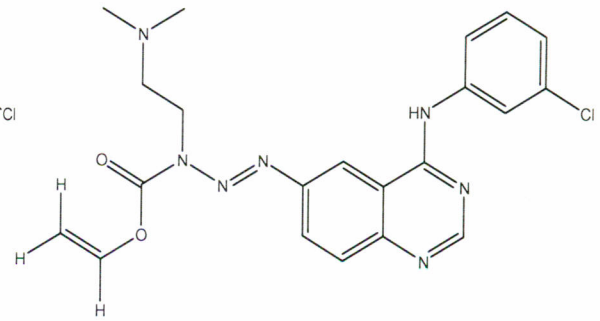
Figure. Structure of RB107 (X=Cl) and RB24 (X=Br). REFERENCE

1.6.3. Introduction to Current Study

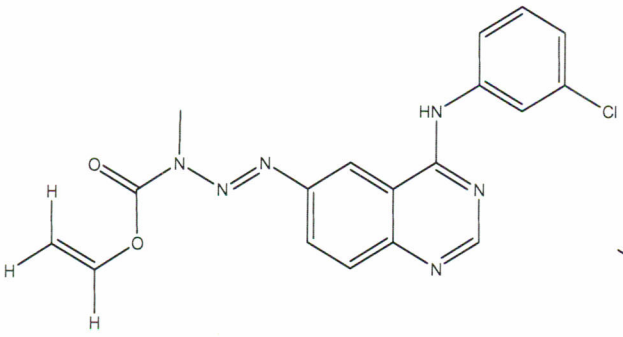
Six molecules were created with modifications to increase the stability. The six molecules presented herein are ZRS1, ZRL1, ZRL2, ZRL3, ZRL4, and ZRL5 (Figure X). This new series of molecules differ from RB107 due to the fact that they are carbamates rather than an acetoxy methyl. This particular modification was chosen because carbamates are known to be generally more stable. As well, each molecule differs from one another by their different functional groups associated with the carbamate. The functional groups were all chosen based on the knowledge that they would decrease the goodness of the leaving group and increase the stability of the molecule. ZRS1 is the most similar to RB107 because it is an acetoxy methyl carbamate. ZRL1 and ZRL2 are vinyl carbamates. ZRL3 and ZRL4 are chloroalkyl carbamates. Finally, ZRL5 is a p-nitro phenol carbamate. Based on their structures we can predict the comparative stability of the molecules. We expect ZRL1 to degrade the fastest due to its dimethylamino etheyl group, which causes internal catalysis of the carbamate to produce an active, fluorescent quinazoline moiety, FD105, and an alkyldiazonium species. (Scheme__)Should I put in the chemical degradation scehemes I created using ChemDraw? This is the only molecule that has the ability to internally catalyze its degradation, the rest of the molecules degrade via nucleophilic attack by water. (Schemes __to__) It is important to note that this method of degradation is only the predicted chemical degradation and does not take into consideration the many enzymes present in cells that could affect the degradation of the molecules producing unpredicted intracellular degradation results.



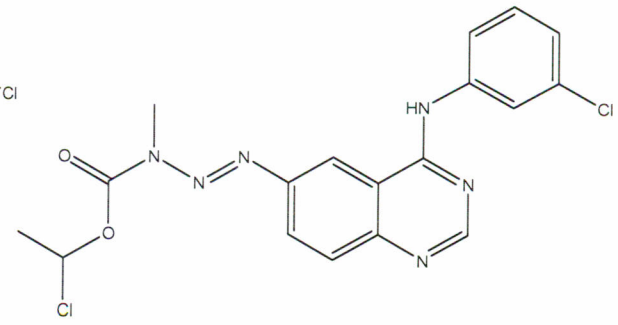
ZRS1



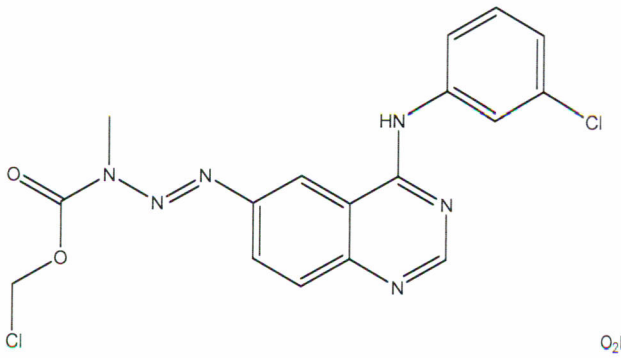
ZRL1



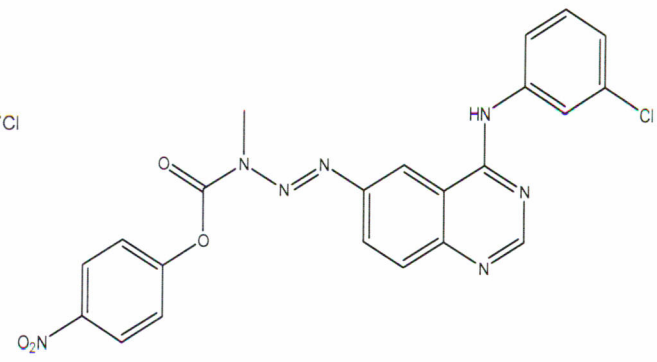
ZRL2



ZRL3



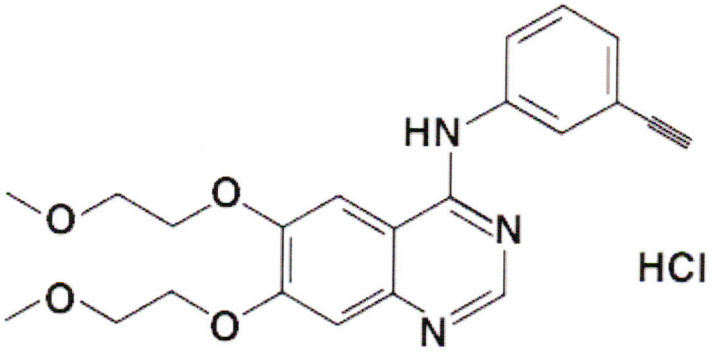
ZRL4



ZRL5

Figure Structure of new series of molecules: ZRS1 (top left), ZRL1 (top right), ZRL2 (middle left), ZRL3 (middle right), ZRL4 (bottom left), and ZRL5 (bottom right).

We predicted that we would not cause a significant decrease in efficacy of these molecules compared to RB107 because the modifications were made to a part of the molecule that is not involved in the interaction with the ATP binding site. The crystallography of erlotinib, which has a similar structure to our molecules, bound to the ATP site provides an example. The image in Figure X shows that the part of erlotinib where our modifications were extends outside of the pocket.



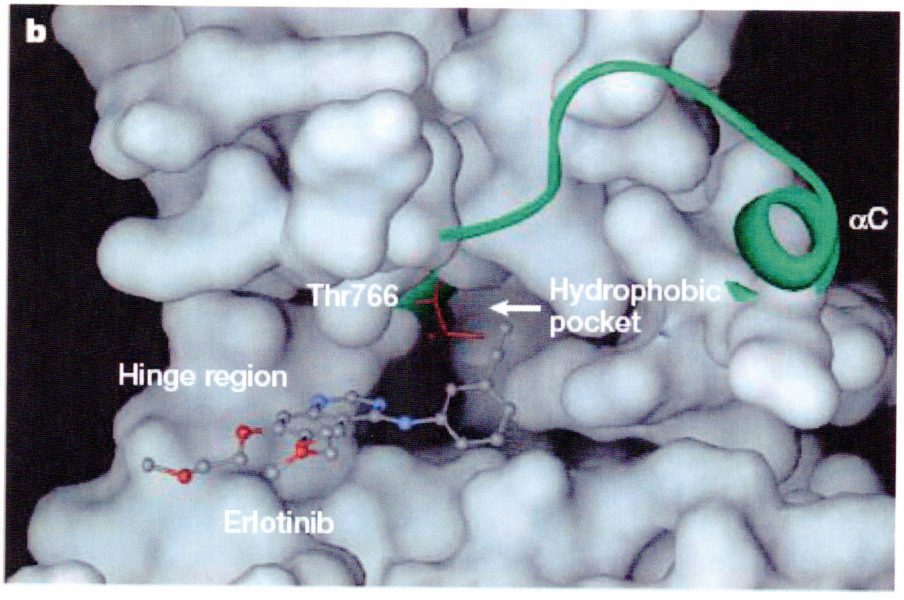


Figure. Structure of Erlotinib (top). Crystallography of erlotinib bound to the ATP binding site on EGFR (Daub, *et al.*,2004). The Red atoms outside of the pocket are the oxygen atoms.

1.7. Research Objectives

1.7.1. Statement of Purpose

Modern cancer drug research is confronted with two major problems: a) lack of sensitivity of current tumour regimens and b) tumour resistance to the current therapies. To circumvent these problems, we recently developed a novel approach termed combi-targeting that seeks to synthesize chimeric molecular combinations designed "combi-molecules" capable of targeting tumour specific growth factor receptors (selectivity components), blocking their mitogenic signalling, and simultaneously damaging DNA. Drug development within the framework of the combi-targeting concept was significantly hampered by the instability of the combi-molecules. To circumvent this problem we designed a series of combi-molecules masked with different functional groups (e.g. acetoxy, p-nitrobenzene, vinyl ester) designed to delay their hydrolysis. My project seeks to achieve three major aims: 1) study the kinetic degradation of this series of combi-molecules; 2) demonstrate their mixed EGFR TK inhibitory and DNA damaging properties; 3) select one compound on the basis of stability and binary EGFR-DNA targeting potency. This project will lead to the development of a novel agent for the therapy of refractory tumours.

1.7.2. Contribution of Authors

All of the experiments in this thesis were conducted by me with the exception of the synthesis of ZRS1, ZRL1, ZRL2, ZRL3, ZRL4, and ZRL5, which was done by Dr. Zakaria Rachid. The writing was completed under the guidance of my supervisor, Dr. Bertrand J. Jean-Claude.