

Review Article

Translational Research in Cancer Drug Development

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Abstract

The development of cancer treatment were initiated by the existence of human's effort to treat by applying certain materials which is mostly part(s) or extracts of plants, which are now adapted as traditional herbal medicine. The discovery of new drugs was based on intuition and empirical evidence. Thus, high luck factor was involved in a successful treatment with unguaranteed reproducibility. One example of drug being developed through conventional drug development is Taxol. Taxol is an extremely complex natural product and requires a bunch of hard work with high level of serendipity to be discovered as antitumor agent. Recently, rapid development in human biology and technology allow a change in drug discovery strategy by minimizing the luck factor. Targeted therapy has been a very promising strategy of drug development research, especially in cancer treatment. Although cancer has been known as a disease with very complex cellular and histo-pathophysiology, the abundance of studies on proteins, such as receptors and hormones, as the hallmarks of cancer allows us to explore carcinogenesis suppression further based on molecular targeted therapy. Kinases, one type of protein involved in signal transduction regulating cell growth and differentiation, could be the proteins that are proposed to be inhibited in suppressing tumor growth. An interesting example of the drug being discovered based on molecular modeling is the discovery of lapatinib as anti-cancer with specific target on HER-2 and EGFR to overcome the resistance of cancer to Herceptin caused by elevated level of EGFR expression.

Key words : targeted therapy, cancer, translational drug development

INTRODUCTION

Cancer is a disease with a molecular cause of which vary according to each type. An alternative therapeutic target is the inhibition of proteins involved in signaling processes leading to the growth and development of cancer cells and the proteins involved in the resistance mechanisms of cancer. Systemic chemotherapy on cancer shows less effectiveness and selectiveness. It has toxic effects on normal cells, too.

Breast cancer, a sample of major death cause, is the most common cause of cancer death in several decades. The molecular mechanism of breast cancer is not fully understood, but it is possibly related to the expression of oncogenes such as c-myc, ERBB2, and Ras (Bouker *et al.*, 2005). Breast

cancer is also caused by mutations of BRCA1 (breast cancer, type 1), BRCA2 (breast cancer, type 2), and p53 gene (Kumar *et al.*, 2000). The other possible mechanism is the inactivation of p53 wild-type resulting in the loss of function as a tumor suppressor gene (Hanahan and Weinberg *et al.*, 2011). The failure of breast cancer therapies are mostly caused by resistance or multidrug resistance. This phenomenon is mediated by breast cancer resistance protein (BCRP), such as P-glycoprotein (Pgp) (Imai *et al.*, 2004). Thus, developing a specific drug for resistant breast cancer therapy to reduce death of breast cancer patients is such an urgent issue.

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Conventional drug discovery began with virtually the same methods belong to the early antibiotics. Bioassays were used to screen large libraries of natural products, phytochemicals, or synthetic organic compounds to find a few bioactive chemicals. We could barely find compounds derived from screening to be directly useful as anticancer drugs. The candidate must be elaborated to a drug through repetitive synthesis of chemical analogs to improve its specificity, potency, bioavailability, and safety. Many years needed to elaborate a screening drug candidate into a preclinical drug development candidate.

Nevertheless, most candidates failed to be synthesized in an acceptable amount and accurately predict key aspects of drug behavior in human clinical trials. As a result of the high failure, the cost of drug development is getting higher and reach \$10 million budget per drug item (Matsumoto, 2008). The cancer therapy has been switched to molecular targeted therapy that is based on protein library and chemical library (synthetic and phytochemicals) (Lee *et al.*, 2011). Molecular targeted therapy ensures more effectiveness and efficiency since only tumor cells, not the normal cells would be affected. Specific drug for molecular targeted therapy discovery is important to be the focus of cancer drug development. And the resistance being involved in molecular targeted therapy would be also a focus in order to overcome resistance problem.

Conventional Drugdevelopment

Indonesia, as one of "megabiodiversity" countries of the world, possesses a huge number of bio-resources which can be applied to a wide range of natural products, such as herbal/traditional medicine for cancer therapy as both as first-line drug of therapy and as complementary drug of conventional chemotherapeutic agents in combinational therapies. Drug development in Indonesia started with the

applications of traditional medicine. Currently, both natural products chemists and phytochemists believes that the secondary metabolites contained in natural products are mostly used in the purpose of mostly self defense against harmful effects of toxins, carcinogens, or mutagens found in the plant (Mitscher *et al.*, 1986) or attack by external predators (Woodbury *et al.*, 1961). Pharmacological tests was done to proof their activity, starting from collection of samples, extraction, bioassay, pre-clinical study, formulation, until finally clinical trials are conducted.

One example of drug being developed through conventional drug development is Taxol. Having the mechanism of antitumor activity through binding to a protein, tubulin, causing the inhibition of cell division, taxol has been called as the best new anticancer agent being developed from natural products, showing efficacy against ovarian cancer (Wall and Wani, 1996), even being referred as 'the most important new drug we have had in cancer in 15 years by NCI (Kolata, 1991). Taxol is an extremely complex natural product, possessing the molecular weight of 853, forming the compound $C_{47}H_{51}NO_{14}$ (figure 1) (Wani *et al.*, 1971). Just like many previous investigations of biocompounds' activity, the discovery of this antitumor agent requires a bunch of hard work with high level of serendipity (Wall and Wani, 1996). In the early 1960s, as part of the NCI–United States Department of Agriculture (hereafter USDA) plant screening program, taxol was found to be present in the bark of the Pacific yew (*Taxus brevifolia*), a slow growing tree mainly in the Pacific Northwest of the United States, in Oregon and Washington. Their development into clinically active agents lasted for about 30 years, from the early 1960s to the 1990s (figure 2) (Cragg and Newman, 2005).

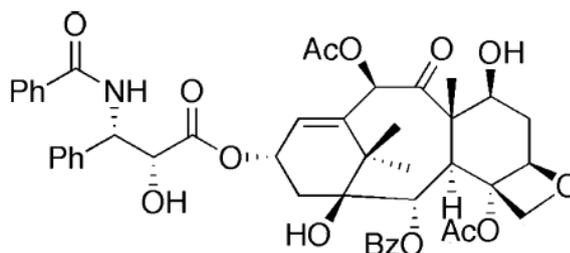


Figure 1. Structure of Taxol

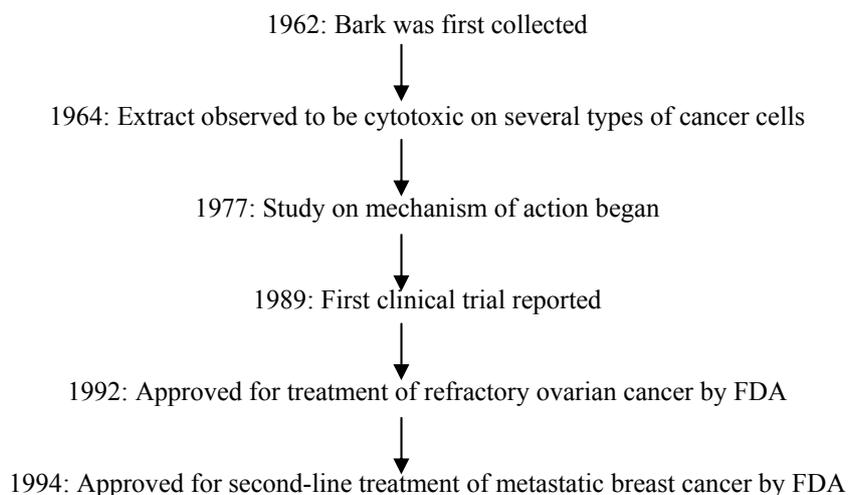


Figure 2. Development of Taxol as chemotherapeutic agent

The bark was first collected in 1962, then the extract was found to be strongly cytotoxic against several types of cancer cells two years later (Walsh and Goodman, 1999). The study of the mechanism of action of this molecule was initiated in 1977 (Horwitz, 2004), and taxol bioactivity was then demonstrated at first in 1979, and the first results of clinical trials were reported a decade later, followed by the announcement of taxol's potential in the treatment of ovarian cancer to the public (Suffness, 1994). FDA approved taxol as an effective chemotherapeutic agent against a wide range of tumor in 1992 (Kohler and Goldspiel, 1994). In 1998, taxol was approved for use against ovarian and advanced breast cancer in many parts of the world, even has been the first-line drug of choice in ovarian cancer treatment; and is potential to be used against other types of cancer. Nowadays, taxol is

widely used in about one fourth of cancer treatment incidence.

Following the announcement of taxol's potential as anticancer agent, controversies came along. The issue of developing cancer chemotherapy versus the issue of environmental protection came up, since stripping the bark from the yew killed the tree (Walsh and Goodman, 1999). Between 1974 and 1989, NCI had extracted bark from about 7500 trees (Snader, 1990) mostly for investigative research. After 1984, when taxol entered phase I clinical trials, the order increased gradually. Until at least the end of 20th century, this tree was still the main source of the molecule (Walsh and Goodman, 1999), with the yield of only about 0.004%, since approximately 0.5 g of taxol isolated requires around 12 kg of air-dried stem and bark (Wall and Wani, 1996). While a full course of treatment for a patient may require 2 g of taxol, being administrated several times

over many months (Strobel *et al.*, 1996). Rendering to those facts, the synthesis of taxol was then developed.

Although it was around the end of the 20th century when the semisynthetic method (conversion of baccatin III or 10-deacetyl baccatin III) obtained from *Taxus* spp. to taxol) (Stierle *et al.*, 1994; Denis *et al.*, 1988) and total synthesis was introduced (Nicolau *et al.*, 1994), problem remained, since those multistep processes seemed uneconomical. In order to lower the price of taxol, fermentation process was then developed. The discovery of *Taxomyces andreanae* (organism other than *Taxus* spp. being able to produce taxol) was the first to be demonstrated (Stierle *et al.*, 1994). Unfortunately, the yields of taxol and taxanes obtained was low. Research on the development of synthesis and biosynthesis technique in obtaining taxol is recently still ongoing to develop more effective routes to this biocompound, together with the investigation of more biologically active and easier to synthesize molecule derived from the molecule (Guo *et al.*, 2006). Furthermore, biotechnological approach was also conducted, in order to establish highly productive cell cultures at the industrial level (Exposito *et al.*, 2009).

The review above shows that the development of anticancer drug which is based on the natural materials led to a serendipitous and exhausting research,

spanning over a long period of time. Thus, anticancer drug development paradigm which is based on the exploration of natural materials led to the slow development of cancer drug discovery in Indonesia. However, previous studies on natural products brings along huge amount of information allowing a rapid development of it through translational researches on targeted therapy, which is promising. Besides, further exploration may also provide scientific basis and targeted therapy approach for the application of natural products that are still widely used, particularly on cancer treatment, both solely and in combination with established chemotherapeutic agents.

Translational Drug Development

Translational research is a concept of research with a shortcut that may shorten the path of new drug discovery. With the basis of bioinformatics-genomics and the abundant information on molecular targeting of pathogenesis biomarker, we may computationally design a molecule predicted to be potentially active and easily synthesized. This model may predict a molecule as potent candidate from genotypic level to then brought into phenotypic level (figure 3).

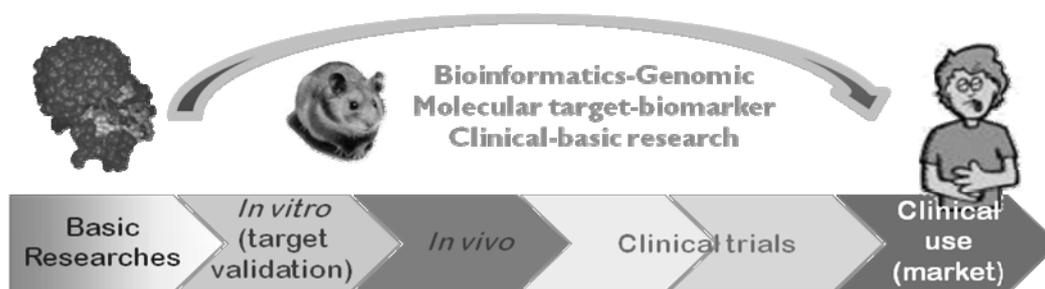


Figure 3. Basic concept of translational research, by analyzing interactions in genotypic level to predict phenomenon in phenotypic level, giving the chance to skip several high-failure-risky steps of drug development.

Identification of the protein markers in a specific cancer is essential for developing chemical library of molecules that are potential to have specific interactions on

them. The specific interaction of receptor and its ligands is expected to modulate the normal effect of the protein. This model of interaction ensures more effectiveness of the

agent when administrated solely and may decrease the side effect caused by chemotherapeutic agents when applied in combination. This strategy is effectively supported by the increasing techniques in drug discovery based on molecular modeling employing computer modeling based drug design. An interesting example of the drug being discovered based on molecular modeling is the discovery of lapatinib as anti-cancer with specific target on HER-2 and EGFR to overcome the resistance of cancer to Herceptin (Konecny *et al.*, 2008).

HER-2, or HER-2/neu or Erb-B2 receptor, is a member of the EGFR superfamily, a tyrosine kinases transmembrane receptor. This growth factor receptor influences the growth, survival, metastasis, invasiveness, and angiogenesis of tumor cells (Laskin *et al.*, 2004). Since HER-2 is expressed as the hallmark of several cases of malignant breast cancer, such receptor has been a promising target in breast cancer. Thus, HER-2 becomes the important target in the inhibition of cell proliferation. One drug that is popularly used which targets HER-2 receptor is Herceptin.

Herceptin is clinically effective for breast cancer with HER2 over expression, but breast cancer patients develop resistance in prolonged used, caused by elevated level of EGFR expression. Therefore, EGFR is an important target in breast cancer drug development. Gefitinib (figure 4) is a small molecule given orally and works by blocking the mechanism of EGFR at the ATP binding site, thereby inhibiting proliferation, differentiation, and angiogenesis (Birnbau *et al.*, 2005). On the other hand, gefitinib causes resistance and secondary mutation (Yang *et al.*, 2005). To overcome the secondary mutation caused by gefitinib, one has developed WZ4002, a compound that targets EGFR without causing other resistance mechanism. WZ4002 is selective to mutated EGFR with no effect on wild type EGFR (Zhou *et al.*, 2009). Therefore, unless some efforts are taken to overcome the phenomenon, there would be no effective treatment to breast cancer remains.

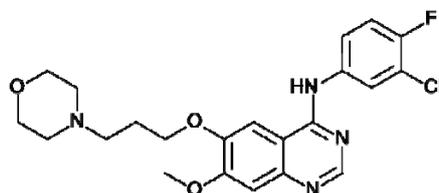


Figure 4. Chemical structure of Gefitinib

EGFR family has four members that their binding with proper ligands lead to the activation of signaling cascades involved in cell proliferation and survival (Nahta *et al.*, 2003; Moy and Gross, 2006). Although ligands for HER-2 are not known, it plays a role in EGFR-family signaling through heterodimerization with other members of the family (Rusnak *et al.*, 2001). Since the overexpression of EGFR and HER-2 are the hallmarks of some cases of breast cancer, agents inhibiting both EGFR and HER2 might be more effective against cancer than those targeting either receptor alone. This idea led to the development of lapatinib (Moy *et al.*, 2007).

Lapatinib (figure 5) is a tyrosine kinase inhibitor that works on both EGFR and HER2 tyrosine kinase domains (figure 6) (Moy and Goss, 2006; Rusnak *et al.*, 2001; Konecny *et al.*, 2006). Not only showed potent activity against cancers with overexpression of EGFR and HER2, the drug is active against cell lines that are resistant to herceptin, thus promising in patients with trastuzumab-resistant cancers. Besides, since lapatinib is a small molecule that is able to penetrate the blood brain barrier, it may also overcome the problem of herceptin's disability to treat or prevent brain metastases that develop in about one-third of patients with HER2-overexpressing metastatic breast cancer (Moy and Goss, 2006; Clayton *et al.*, 2004).

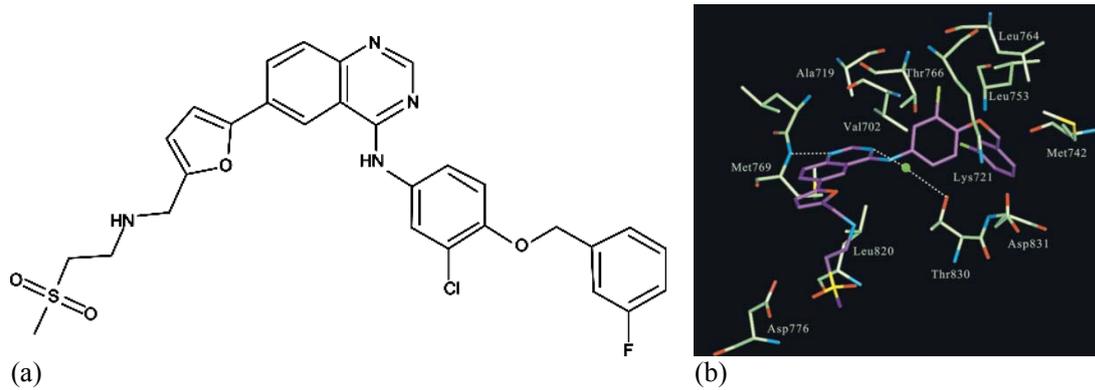


Figure 5. Chemical structure of Lapatinib (a) and its docking with EGFR (Wood *et al.*, 2004) (b)

Structural biology of protein kinase has given detail insights into catalytic domain of kinases to develop selective kinase inhibitor (Cowan-Jacob *et al.*, 2009). Crystal structure of protein explain us binding affinity between kinase inhibitor and receptor. Some computational models have been developed and seem to be useful for ligand scening, ligand docking, and ligand activity profiling studies. Resistance of kinase domain because of mutation contribute to stability of conformation of kinase. By knowing structural

biology of kinase, kinase inhibitor may be designed to overcome the resistance problems. According to those facts, we may see the success and ongoing rapid discoveries with the paradigm of translational research.

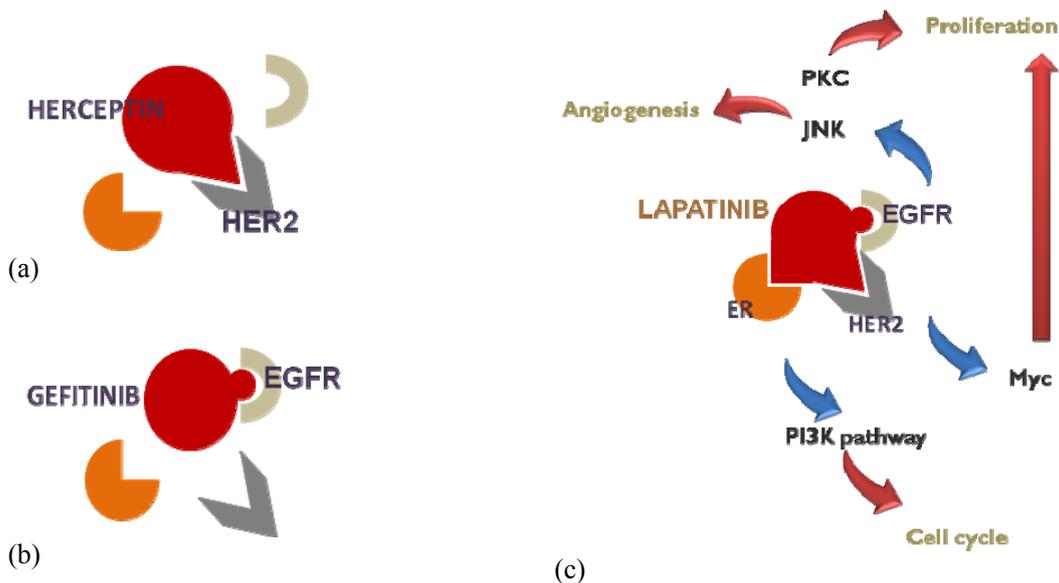


Figure 6. Illustrations of interactions between herceptin and its receptor (a), gefitinib and its receptor (b), and lapatinib possessing triple receptor as targets, along with the downstream proteins involved (c)

Tyrosine kinase inhibitor is one promising approach in developing drug for cancer targeted therapy, since kinases do the protein phosphorylation, thus play an important role in regulating signal transductions involved in proliferation, differentiation, migration, and apoptosis of cells. In some types of cancer, mutation or overexpression of certain tyrosine kinases occur (Broekman *et al.*, 2011). A tyrosine kinase inhibitor, sunitinib, inhibited tumor growth, metastasis, angiogenesis, and cell proliferation in a mouse model of mammary cancer (Tanaka *et al.*, 2011). Sunitinib, which is also known as sunitinib, targets stem-cell factor receptor (KIT or SCFR) and FMS-like tyrosin kinase-3 (FLT3) that are involved in pro-oncogenic pathway and also VEGFR and PDGFR that are known to play a role in angiogenesis signal transduction (Christensen, 2007; Nishioka *et al.*, 2009).

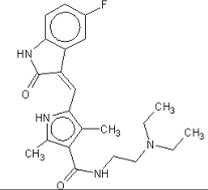
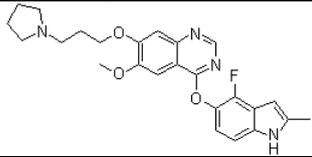
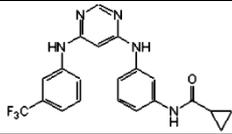
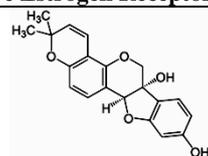
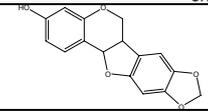
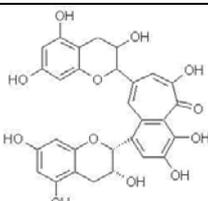
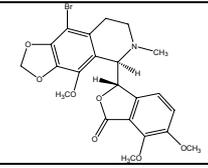
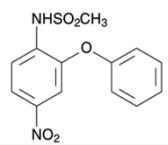
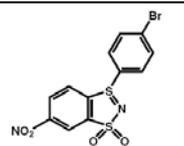
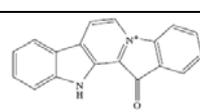
Cediranib is another example of molecule inhibiting tyrosine kinase. This molecule targets VEGFR 1, 2, 3, c-KIT, and PDGFR and shows inhibition on both angiogenesis and growth of several tumor models (Nikolinakos and Heymach, 2008). Proven to impair the proliferation and migration of endothelial cells along with their ability to form tubes *in vitro*, cediranib also reduced angiogenesis, tumor perfusion, and inhibited growth of Caki-1 tumor cells-induced angiogenesis *in vivo* (Siemann *et al.*, 2009). Another data showed that cediranib shows potent inhibition activity on pan-VEGFR kinase with the activity similar to the one on KIT, but less than the ones on PDGFR- α and PDGFR- β (Brave *et al.*, 2011). A molecule coded as 324674 was proven to be EGFR-specific in inhibiting infection of HeLa cells compared to CHO cells (Mercer *et al.*, 2010). This compound irreversibly inhibit EGFR activation and the downstream, AKT signaling pathway, in a dose-dependent manner (Yu *et al.*, 2011).

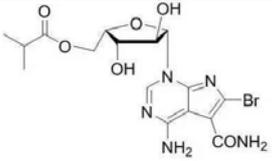
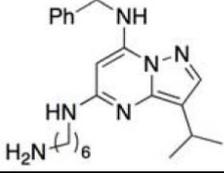
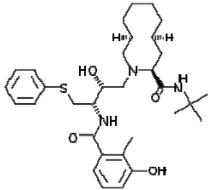
The steroid hormone estrogen plays an important role in the development and differentiation of breast and endometrial cells, thus may have certain effects in the growth of cancer, either directly by activating oncogenic pathways or indirectly via mitogenic stimulus (Glaeser *et al.*, 2006). However, estrogen also plays an important role in maintaining bone density, cholesterol level, and reproductive

tissues (Jordan, 2004). Those effects are mediated by specific receptor, that is estrogen receptor (ER). Hence, ER targeting is one approach of both chemoprevention and cancer targeted therapy (Manni *et al.*, 2011). Glyceollin I, a compound isolated from activated soy, showed inhibition on ER-mediated gene expression and cell proliferation of MCF-7 breast cancer cells and BG-1 ovarian cancer cells *in vitro* (Zimmermann *et al.*, 2010). Docking result showed that glyceollin I binds ER α with type II antiestrogenic binding (Zimmermann *et al.*, 2010). Observation on estrogenic effect of glyceollin by using E-screen assay and pS2 expression showed that this compound had higher affinity to ER β than ER α , whilst *in vivo* study proved that glyceollin increased breast proliferation and the markers of ER activity in breast epithelium, thus possessing estrogenic effect (Wood *et al.*, 2006). Pterocarpan, a group of natural isoflavonoids showing potent antitumor and antimetabolic activity, could increase uterine weight of young female rats, thus possesses estrogenic effect (Abdel-Kader, 2010).

There are two anticancer mechanism of actions involving tubulin, that are by promoting or by inhibiting tubulin polymerization. Tubulin itself is a protein assemblies and disassemblies in cell replication, thus any interference to it will lead to cell division disruption (mitotic block) and cell death (apoptosis) (Jordan and Wilson, 2004; Kingston, 2009). Theaflavin, belonging to the polyphenols, acts as microtubule depolymerizers in HeLa cells and inhibits polymerization of purified tubulin (Chakrabarty *et al.*, 2011). Whilst EMO-11, a 9-bromo derivative of noscapine, stabilizes microtubules in an idle state, decreasing microtubule dynamics, leading to cell death (Aneja *et al.*, 2010). Not only being nonimmunosuppressive *in vivo*, this compound also showed more potent anti cancer activity and less toxicity compared to noscapine itself (Joshi *et al.*, 2009).

Table I. Potential Molecular Targeted Anticancer

Compound	Molecular structure	Activity	Ref.
Tyrosine Kinase Inhibitor			
Sutent		Inhibits SCFR, FLT3, VEGFR, PDGFR	Christensen, 2007 Nishioka <i>et al.</i> , 2009
Cediranib		Inhibits VEGFR1, VEGFR2, VEGFR3, c-KIT, PDGFR	Nikolinakos and Heymach, 2008 Siemann <i>et al.</i> , 2009 Brave <i>et al.</i> , 2011
324674		Inhibits EGFR	Mercer <i>et al.</i> , 2010 Yu <i>et al.</i> , 2011
Anti-estrogen and Selective Estrogen Receptor Modulator			
Glyceollin		Estrogen agonist and antagonist	Wood <i>et al.</i> , 2006 Zimmermann <i>et al.</i> , 2010
Pterocarpan maccian (Mac)		Estrogenic effect	Abdel-Kader, 2010
Tubulin binding			
Theaflavin		Inhibits tubulin polymerization	Chakrabarty <i>et al.</i> , 2011
EMO-11		Decreasing microtubule dynamics	Joshi <i>et al.</i> , 2009 Aneja <i>et al.</i> , 2010
COX-2 inhibitor			
Nimesulide		Inhibits the activity of COX-2	Hida <i>et al.</i> , 2006
CX-9051		Inhibits the activity of COX-2	Huang <i>et al.</i> , 2010
CDK inhibitor			
Fascaplysin		Inhibits the activity of CDK4	Lin <i>et al.</i> , 2007

Ibulocydine		Inhibits the activity of CDK7 and CDK9	Cho <i>et al.</i> , 2011
BS-181		Inhibits the activity of CDK7	Ali <i>et al.</i> , 2009
Protease Inhibitor			
Nelfinavir		Inhibits the activity of proteases	Gills <i>et al.</i> , 2007

Cyclooxygenase-2 (COX-2) is important enzyme in inflammation and carcinogenesis. Expression of COX-2 stimulates the occurrence of cancer by increasing prostanoid production that increases cancer cell proliferation (Kinoshita *et al.*, 1999). Upregulation of COX-2 causes upregulation of Bcl-2 that leads to apoptosis resistancy (Sheng *et al.*, 1998) and causes excessive accumulation of prostaglandins (PGH₂) which catalyzes the conversion of procarcinogen to be carcinogen (Palozza *et al.*, 2004). Inhibition of COX-2 may help treat and prevent cancer.

The first generation of COX-2 inhibitor as anticancer is celecoxib. The other COX-2 inhibitor anticancer drug is nimesulide. COX-2 expression in NSCLC cells may affect their responsiveness to COX-2 inhibitors, so the apoptosis induction of nimesulide on non-small cell lung cancer cell is correlated to inhibition of COX-2 expression (Hida *et al.*, 2006).

CX-9051 is new generation of COX-2 inhibitor developed based on computational strategy. CX9051, a benzo[1,3,2]dithiazolium ylide-based compound, shows inhibition COX-2 activity (IC₅₀ 1.3 μM) with similar efficacy to activity of celecoxib (IC₅₀ 0.47 μM) (Huang *et al.*, 2010). CX9051 shows apoptosis induction by modulation of NF-κB translocation and protein expression and induces TRAIL up-regulation and activation

of extrinsic apoptotic signaling of PC-3 and DU145 cells (Huang *et al.*, 2010).

Cell cycle is regulated by cyclin-dependent kinases (CDK), which are activated during the cell moves from G1 to S phase into G2 and M phases, which form a complex with cyclin. CDK activity is controlled by cell cycle inhibitor proteins known as CDK inhibitors (CKI). Development of CDK inhibitors as anticancer agents has been interesting topic. There are some CDK inhibitors approved by FDA as anticancer such as roscovitine and flavopiridol. Fascalypsin is one of new generation of CDK inhibitors. Fascalypsin, a red pigment originally isolated from Fijian marine sponge *Fascaplysinopsis* sp. in 1988, represents the Wrst naturally occurring member of the pentacyclic ring system 12H-pyrido [1, 2-a: 3,4-b']diindole. Study of fascaplysin on chorioallantoic membrane (CAM) model and human umbilical vein endothelial cell (HUVEC) line shows that fascaplysin inhibits of new blood vessel formation at the tumor site other than killing tumor cells directly with selective CDK4 inhibition activity (Lin *et al.*, 2007).

Previous study work on CDK inhibitor is still ongoing. BS-181 is a synthetic compound developed based on computational drug development. BS-181 shows strong CDK inhibition activity especially CDK7, induces cell cycle arrest

and apoptosis of cancer cells, and shows in vivo antitumor activity (Ali *et al.*, 2009). Ibuloctidine (an isobutyrate prodrug of the specific CDK inhibitor, BMK-Y101), a candidate anti-cancer drug for HCC, inhibits HCC cells proliferation and induces apoptosis by inhibiting CDK7 and CDK9 (Cho *et al.*, 2011).

Protease inhibitors perturb replication of HIV (human immunodeficiency virus) and interfere with the protease enzyme. Blocking of protease leads to aberration of the new copies of HIV formation. Nelfinavir is one HIV protease inhibitor that is developed as anticancer. Development of nelfinavir as anticancer is directed by toxic effect of nelfinavir associated to inhibition of Akt. Nelfinavir induces cell death by caspase dependent and independent pathway, decreases the viability of drug-resistant breast cancer cell lines and inhibits NSCLC xenografts growth that is associated with

induction of ER stress, autophagy, and apoptosis (Gills *et al.*, 2007).

The abundance of information on the activity and molecular targets of chemicals particularly in the treatment of cancer, supported by studies on receptors being involved in carcinogenesis allows a promising translational research with the basis of cancer targeted therapy. Computational study resulting prediction of new active molecules may bring along an effective and efficient discovery and development of new drugs possessing anticancer activity. Besides, further and comprehensive studies on targeted therapy let us conduct a molecular approach in finding the scientific basis of natural products application (as traditional medicine). The focus of drug development in Indonesia should be immediately turned, from the development of herbal products to the development of modern medicine.

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