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REVIEW ARTICLE

Recent Updates on the Therapeutic Potential of HER2 Tyrosine Kinase Inhibitors for the Treatment of Breast Cancer

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Abstract: HER2 positive breast cancer is characterized by the low survival rate in the metastatic patients. Development of resistance and disease-relapse are the major problems associated with the currently available therapies for HER2 positive breast cancer. There are two major targeted therapies for HER2 positive breast cancer *viz.* monoclonal antibodies and tyrosine-kinase inhibitors, and both of these therapies have their advantages and limitations. To address the limitations associated with the existing therapies, use of antibodies and TKIs as combination therapy proved to be more effective. Various chemical modifications can be performed on tyrosine-kinase inhibitors to develop novel ligands with increased selectivity for HER2 kinase. A number of tyrosine-kinase inhibitors are in various phases of clinical trials for the treatment of HER2 positive breast cancer. In the current review article, recent developments on various HER2 tyrosine-kinase inhibitors have been reported. Various structurally different scaffolds bind to the HER2 receptor and exhibit potent anti-cancer activities. The structural and pharmacophoric requirements of the scaffolds are discussed in detail so as to discover effective drug candidates for the treatment of HER2 positive breast cancer.

Keywords: Breast cancer, HER1/HER2, Tyrosine kinase inhibitors, Drug resistance, Monoclonal antibodies, Trastuzumab.

1. INTRODUCTION

Worldwide breast cancer is the most-frequently occurring malignancy among women accounting for about 23% of total cancer cases and 14% of the mortalities [1]. The incidences of breast malignancy and the mortalities have also been reported among men [2]. Expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) determines the clinical subtype of breast cancer. The biological behavior and sensitivity towards treatment differs for each of the three major clinical subtypes of breast cancer *i.e.*, the hormone receptor-positive (HR+; ER- and/or PR-positive), HER2-positive (HER2+ and/or HR+), and the triple-negative (TN; negative expression of ER, PR and HER2) [3]. Almost 15-30 % of the breast cancer cases are HER2-positive, with HER2 gene amplification or overexpression of HER2 protein [4]. In HER2 positive tumor, an enormous increase in HER2 receptor expression (about 2 million receptors) at the tumor cell surface has been observed in some patients [5].

HER2 (ErBb2, neu, p185) is a tyrosine kinase (TK) protein encoded by neu/erbB2/c-erbB2 oncogene located at the long arm of chromosome 17q (17q12) [4, 6, 7]. It belongs to epidermal growth factor receptor (EGFR) family encompassing four TK receptor proteins *viz.*, HER1/EGFR, HER2, HER3 and HER4. HER2 plays a key role in the development of normal and malignant breast tissue [8-9]. Aberrant activation of HER1/HER2 overexpression is associated with aggressive phenotype, *i.e.*, increased chances of relapse and decreased survival [10, 11]. HER2 positive cancers exhibit distinguished biological and clinical behaviour such as high histologic grade [12], increased potential of metastization to brain and viscera and increased resistance towards anti-HER2 agents [5]. Homodimerization or heterodimerization of HER2 with other EGFR family members prompts activation of a multitude of cell-signaling pathways eventually accelerating cellular signaling, proliferation, cell-cycle degradation and decelerating apoptosis [4, 7, 8, 11].

Homodimerization of HER2 takes place independent of ligand and this makes HER2, unique member of EGFR family [13]. Heterodimers of HER2 own most-robust signaling activity as a consequence of strongest catalytic kinase activity of HER2. Moreover, amongst other constituents of EGFR family, the open conformation of HER2 makes it a favourable dimerization partner. HER3 is deprived of TK activity and forms the most-potent heterodimer with HER2, stimulating the anti-apoptotic phosphoinositide 3-kinase, PI3K/Akt pathway. HER2-mediated cell signaling can also be induced upon interaction with insulin-like growth factor receptor-1(IGFR-1) and ER [14]. Thus, HER2 represents itself as an appropriate target for anti-cancer drug development.

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Monoclonal antibodies and tyrosine-kinase inhibitors (TKIs) are the two key classes of HER2 targeting therapeutics. In addition, antibody-drug conjugates have also been developed as the antibody modified drugs for the treatment of HER2 positive breast cancer [15]. Monoclonal antibody, trastuzumab has remained the most successful, for HER2 targeting. However, the development of resistance and cardiotoxicity are the major limitations reported with trastuzumab [16]. TKIs though less selective were found effective to target different cell-signaling pathways in breast cancer and hence lessen the chances of eventual drug resistance development due to cross-signaling [16]. TKI such as lapatinib can be used to treat brain metastases [17]. TKIs can also target trastuzumab-resistant truncated HER2 protein [18]. The clinical profile of TKIs didn't meet the expectation of complete inhibition of HER2-HER3 signaling. Hence, the use of TKIs as monotherapy still remains a challenge [15].

2. HER2: CRYSTAL STRUCTURE OF THE INTRACELLULAR KINASE DOMAIN

Aertgeerts *et al.* [19] reported the first high-resolution crystal structure of the kinase domain of HER2 in complex with SYR127063, a pyrrolo[3,2-d]pyrimidine-based potent and selective HER2 inhibitor. A Gly-rich region (Gly776-Ser779) of α-helix C-β4 loop following α-helix C is unique to HER2, providing conformational flexibility within the HER2 active site. It can be accounted for the previously reported low intrinsic activity of HER2 [19-22]. Genetic analyses on cancer patients have showed that the mutations or insertions of hydrophobic residues before Gly776, enhance the catalytic activity of HER2 [23-26]. A comparative study was also conducted involving the crystal structure of HER1 with TAK-285, a dual HER1/HER2 inhibitor containing pyrrolo[3,2-d]pyrimidine-based backbone so as to develop understanding about the key binding interactions that influences the potency and selectivity of HER inhibitors. The two complexes and their crystal structures were found to be very similar. The kinase domain crystal structure studies revealed that the dimeric protein is asymmetric and each monomer has been occupied by the inhibitor [27]. The HER2 construct taken for structural analysis was a shortened form of human cytoplasmic tyrosine kinase domain, having a part of C-terminal segment. This construct comprises 703–1029 residues and three N-terminal mutations, M706A, Q711L, and M712L were introduced to increase expression as well as to facilitate the crystal formation of the protein-inhibitor complex. A flexible hinge region connects the N-terminal lobe (N-lobe) that contains mostly β-strands and one α-helix; and the C-terminal lobe (C-lobe) is predominantly α-helical. A deep cleft comprising the ATP binding site separates these two lobes. Most of the residues responsible for the catalytic activity were located in proximity to the cleft which include the glycine-rich nucleotide phosphate-binding loop (Leu726–Val734), the α-helix C (αC; Pro761–Ala775) of the N-lobe of the kinase and DFG motif (Asp863–Gly865), the catalytic loop (Arg844–Asn850), and the activation loop (A-loop; Asp863–Val884) of the C-lobe of the kinase. The established feature of the active conformation, *i.e.* the salt bridge interaction between two highly conserved residues (Lys753 and Glu770) has not been observed in the crystal structure. The key binding interactions in the ATP binding site and other features like disordered A-loop were highly similar to that observed for HER1-TAK285 complex. Moreover, HER2-SYR127063 salt bridge formation was also not observed for HER1-TAK-285. The central pyrrolo[3,2-d]pyrimidine ring of SYR127063 exhibits H-bonding interactions with amino-acid residues Met801 and Thr862 and hydrophobic interactions in the adenine site. Hydrophobic interactions with amino-acid residues Thr862, Glu770, Met774, Ser783, Leu785, Leu790, Leu796 and Phe864 were displayed by the trifluoromethyl group of SYR127063 [19].

Aertgeerts *et al.* [19] revealed an allosteric mechanism of action for HER2 which was similar to the activation mechanisms for HER1 and HER2, as reported previously [28-30]. For HER2, a rotation and translation function similar to asymmetric dimers HER1 and HER4 [28-30] was noticed suggesting an allosteric mechanism of activation. Ala705-Thr718 residues in N-terminus, Pro761-Ala775 residues in α-helix C and Leu790-Ser792 residues in loop between β-strand-4 and -5 of one HER2 monomer were involved in interactions with Pro926-Lys937 residues in α-helix G, Gly938-Pro945 residues in loop between α-helix H and α-helix of other HER2 monomer. Mainly closed hydrophobic intermolecular interactions were formed between Lys765, Leu768, Asp769 and Tyr772 residues in α-helix C of one HER2 monomer and C-lobe of kinase domain of other HER2 monomer [19].

We re-docked SYR127063 with the crystal structure of HER2 kinase domain using docking software Schrodinger, Maestro (Version 9.0). The three-dimensional docking structures (Figs. **1** and **2**) showed active site of HER2 kinase domain and ligand-interaction pattern, respectively. From these re-docked structures, we found that the ligand interaction pattern for binding of SYR127063 with HER2 kinase domain was similar as demonstrated previously by Aertgeerts *et al.* [19].

3. THERAPEUTIC AGENTS TARGETING HER2 POSITIVE BREAST CANCER

HER2 targeting agents may be assorted into two classes, *i.e.* monoclonal antibodies and TKIs [15]. The monoclonal antibodies in clinical practice for HER2 positive breast cancer include trastuzumab and pertuzumab (www.accessdata.fda.gov). Trastuzumab is a recombinant humanized monoclonal antibody and it was the first targeted therapy approved for the treatment of HER2 positive breast cancer [31, 32]. Similarly, pertuzumab is a fully humanized monoclonal antibody that slightly differs from trastuzumab with respect to protein-binding domain [33]. Trastuzumab binds to the extracellular domain IV of HER2 and prevent HER2 homodimerization and activate cell-signaling pathway [30]. On the other hand, pertuzumab binds to the extracellular domain II of HER2, and inhibit HER2/HER3 heterodimerization and prevent anti-apoptotic PI3K/Akt signaling [33]. Both of these drugs bind to the extracellular domain and are associated with the adverse drug reactions including cardiotoxicity. It has been observed that trastuzumab exhibited more cardiotoxicity as compared to pertuzumab [33]. The development of *de novo* resistance against antibody therapy is the major problem encountered in most of the cases [16]. Thus new class of drugs derived from antibodies *i.e.* antibody-drug conjugate have been developed in order to potentiate effective drug targeting. Trastuzumab-emtansine (T-DM1) is

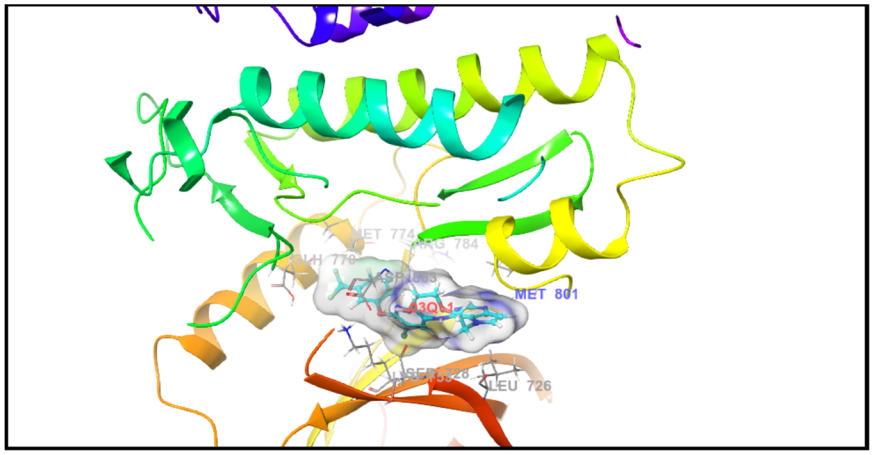

Fig. (1). Active site of HER2 kinase (PDB ID: 3PP0) with co-crystallized ligand SYR127063 (Schrodinger, Maestro 9.0).

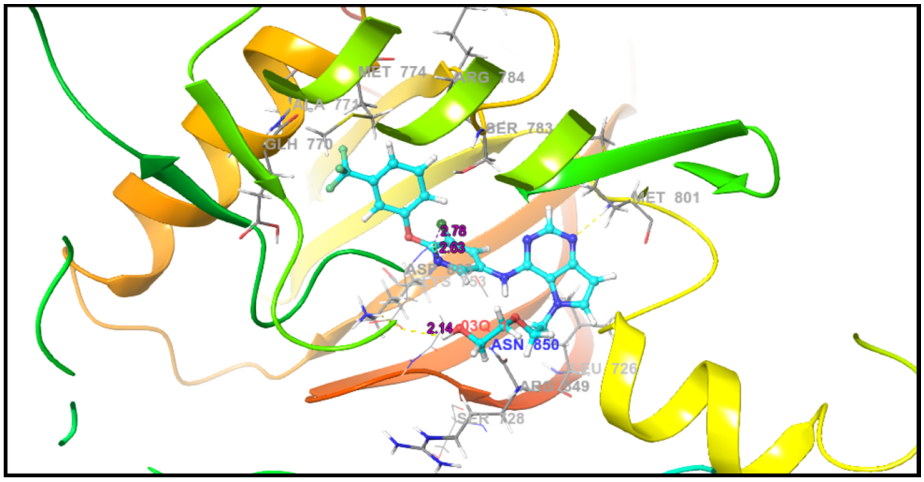

Fig. (2). Expanded view of active site of HER2 kinase (PDB ID: 3PP0) with co-crystallized ligand SYR127063 (Schrodinger, Maestro 9.0).

an approved antibody-drug conjugate for treating breast carcinoma patients with HER2 overexpression (www.access data. fda.gov) [34]. Trastuzumab linked to the microtubule-depolymerizing agent and it facilitates the delivery of cytotoxic agent DM1 to HER2 overexpressing cancer cells [34]. However, the adverse drug reactions remained major issue with this class of drugs too [34].

TKIs are the small-molecules competing for the ATP binding site of the intracellular catalytic domain of kinase. TKI binding inhibit protein phosphorylation and disturb the signal transduction [15]. Lapatinib (GW572016/Tykerb) is the only small-molecule TKI approved by FDA for the treatment of metastatic breast cancer overexpressing HER2 (www.access data. fda.gov). Lapatinib is orally bioavailable and possesses reduced risk of adverse cardiac events as compared to the intravenous administration of antibodies. It effectively inhibits trastuzumab-resistant truncated HER2 protein, p95HER2 [18]. Lapatinib can also cross blood-brain-barrier (BBB) while monoclonal antibodies cannot cross it. Hence, small-molecules may prove effective treatment strategy for curing central nervous system metastases in HER2 overexpressing breast cancer patients [17]. Despite the advantages of lapatinib, development of drug resistance is major concern during treatment [16]. In some cases improved survival rate with T-DM1 has been observed as compared to lapatinib, in HER2 positive breast cancer [35]. The compensatory upregulation of HER3 in response to HER2 inhibition by TKIs mainly accounts for the resistance-acquisition and lower clinical efficacy. Hence, the status of HER2 TKIs as monotherapeutic agents remains controversial. Antibody-based therapy remains the gold standard for the treatment of HER2 overexpressing breast cancer and there are scopes for the improvement of TKIs based treatment strategies with high therapeutic potential. Thus, deep understanding of underlying mechanism of action of TKIs is desired to develop novel drug candidates. In this review article, we have described various developments on the design and discovery of novel HER2 TKIs. In addition, some potent molecules in advance phases of clinical trials are also described.

4. DEVELOPMENTS IN THE SCREENING OF SMALL MOLECULES ACTING AS HER2 TKIS

HER2-targeted antibodies (trastuzumab, pertuzumab) and small-molecule TKIs (lapatinib) are the approved therapies for HER2 positive breast carcinoma (www.accessdata. fda.gov). Trastuzumab exhibits a response rate of only 15-26% among HER2 positive breast cancer patients [36] and in most of the cases it is ineffective as single agent [37]. The development of resistance against antibody-therapy as a result of production of oncogenic truncated form of receptor (p95HER2/HER2Δ16) is the major limitation. Small molecule inhibitors such as lapatinib can inhibit truncated HER2

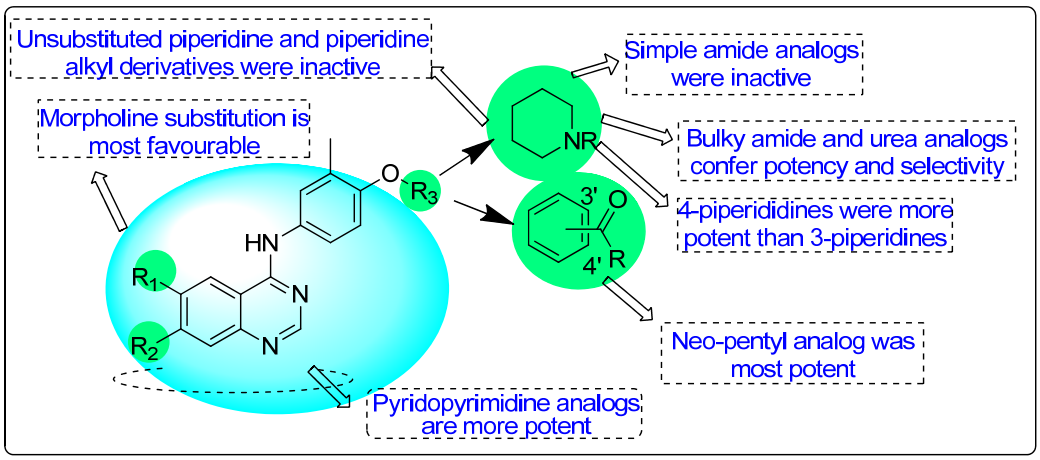

Fig. (3). SAR studies of anilinoquinazolines **(compounds 1-2).**

protein [35]. TKIs can cross BBB, hence these can be developed as effective agents for treating brain metastases in HER2 expressing breast cancer [17]. However, there are some limitations associated with the currently available TKIs such as toxicity, resistance and poor pharmacokinetic profile. Hence, there is need of developing novel HER2 inhibitors with improved pharmacological profile and devoid of above mentioned limitations.

The role of HER2 TKIs for the treatment of breast cancer has been reviewed by a number of research groups [36, 38-44]. Nevertheless, the optimization and structure-activity relationship (SAR) studies have not been discussed in detail. The current review article describes recent optimization studies and novel discoveries of small-molecules as HER2 TKIs. Different HER2 TKIs have been classified on the basis of chemical scaffolds such as 4-anilinoquinazolines, pyrrolotriazenes, pyrrolo and pyrazolo-pyrimidines, thienopyrimidines, and N-aryl pyrimidines.

4.1. 4-Anilinoquinazolines

Lippa *et al.* [45] synthesized a series of selective HER2 kinase inhibitors containing quinazoline and pyrido[4,3-d]pyrimidine scaffolds. The SAR studies for the designed series have been shown in Fig. (**3**). The compounds **1** and **2** (Fig. **4**) were the most potent analogs both inhibiting HER2 kinase and HER2 cells with IC_{50} values below 25 nM. The HCl salt of compound **1** and compound **2** showed low clearance rate of 2.5 ml/min/kg. Compound **1** as HCl salt showed 54% bioavailability while compound **2** exhibited poor bioavailability [45]. Rachid *et al.* [46] designed and synthesized combi-molecules by grafting carbamate-substituted monoalkyltriazene moiety to anilinoquinazolines. These molecules were meant for the inhibition of multiple targets in cancer cells. The synthesized combi-molecules were evaluated as HER1 TK and HER2 transfectant against NIH3T3neu cell line. Compound **3** (Fig. **4**) was found to be the most potent with an IC_{50} value of 1.50 µM. The combi-molecules possessed DNA damaging properties too. SAR studies indicated that the replacement of methyl ethanaoate group with chloromethyl and β-nitrorophenyl groups led to decrease in activity [46]. Mahboobi *et al.* [47] combined the structural features of lapatinib with an (E)-3-(aryl)-N-hydroxyacrylamide motif present in histone-deacetylase (HDAC) inhibitors with an aim to produce multi targeting chimeric compounds inhibiting HER1/HER2 TK and HDAC with less likelihood of developing drug- resistance. Among the synthesized chimeric analogs, compound **4** (Fig. **4**) was found to be the most potent, exhibiting IC_{50} value below 1 µM against HER1/HER2 kinase, HDAC enzyme and HER2 overexpressing SKBR3 cancer cell line. When the furan ring of **4** was replaced with thiophene or benzene ring, the HDAC inhibition activity reduced while HER1 and HER2 inhibition remain unaffected.

The benzamide and hydroxamate analogs obtained *via* replacement of hydroxyacrylamide moiety of **4,** retained the kinase inhibition activity. However, HDAC inhibitory activity diminished in most of the synthesized hybrid analogs [47]. Wu *et al.* [48] synthesized a series of 4-benzothienyl amino quinazolines as hybrids of HER1 inhibitor, gefitinib. The benzothiophene derivatives attribute to increased cytotoxicity but decreased HER1 inhibition than gefitinib. The most potent compound **5** (Fig. **4**) exhibited antiproliferative activity against HER2 transfected MCF-HER2 breast cancer cells with IC_{50} value of 2.7 µM. The secondary amino-substituted propoxy side chain at position 7 in place of position 6 in basic scaffold, led to increased HER2 and MET inhibitory ability with reduced HER1 inhibitory potency [48]. Beckers *et al.* [49] designed and synthesized chimeric broad-spectrum inhibitors of HDAC and HER1/HER2. The linkage of N-(3-ethynylphenyl)quinazoline-4-amine moiety of HER1 inhibitor with an N-(2-aminophenyl)-3-(1-sulfonyl-1H-pyrrol-3-yl)acrylamide motif in erlotinib retained the selective HER1/HER2 kinase inhibitory properties, along with HDAC inhibition. The attachment of N-(3-ethynylphenyl)quinazoline-4-amine moiety with N-(2-aminophenyl)-4-(oxymethyl)benzamidyl group diminished the HDAC inhibitory activity while, significantly improve HER1/HER2 inhibitory activity. In this series, compound **6** (Fig. **4**) was the most potent HER2 inhibitor with an IC_{50} value of 39 nM [49]. Li *et al.* [50] designed and synthesized 6-salicyl derivatives of anilinoquinazolines as HER1/HER2 TK inhibitors. All compounds showed moderate to good *in vitro* inhibitory activities in the enzymatic and cellular assays. Compound **7** (Fig. **5**) showed most potent and selective dual HER1/HER2 inhibitory activity comparable to that of lapatinib, with IC_{50} values of 120/96 nM respectively. From the molecular docking studies, it was established that the two

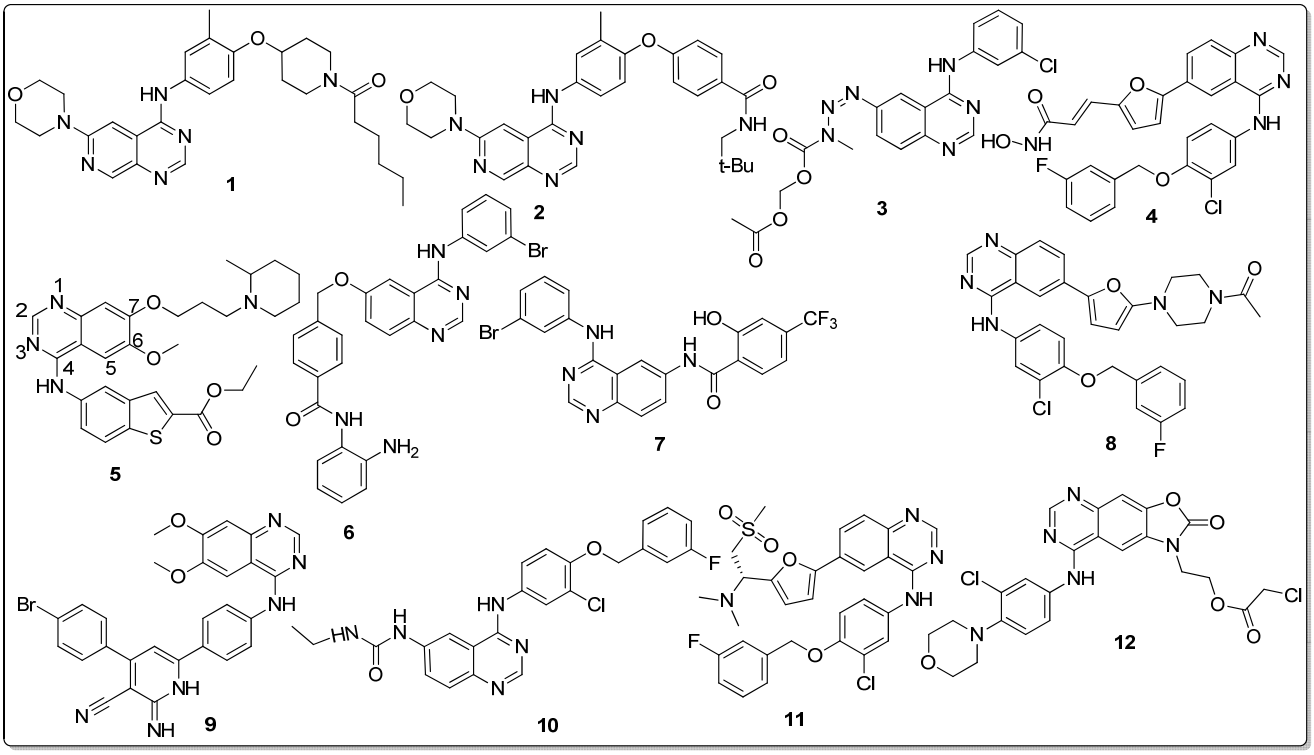

Fig. (4). Structures of anilinoquinazolines acting as HER2 inhibitors.

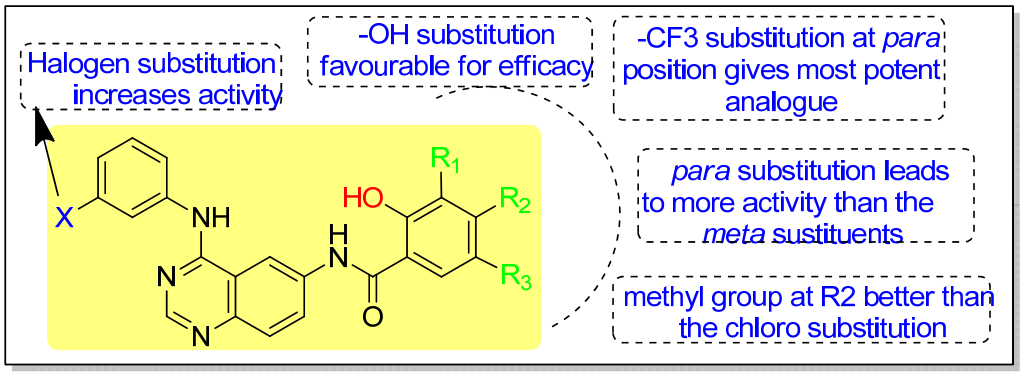

Fig. (5). SAR studies of anilinoquinazolines (compound **7**).

amino acid residues; SER783 and MET801 located in the hinge-binding pocket were crucial for the activity. *Para* substituted trifluoromethyl group, hydroxy substituent, and ketonic oxygen were crucial for the stabilization of the binding complex. Trifluoromethyl group was involved in the H-bonding interactions with PHE731 and ALA730. SAR studies for compound **7** are summarized in Fig. (**5**) [50]. Zhang *et al.* [51] designed and synthesized lapatinib hybrids as potent dual HER1/HER2 inhibitors with improved drug like characteristics. Compounds **8** (selatinib, Fig. **4**) was found to be the most potent in the series displaying selective inhibitory activity with IC_{50} values of 19.2 nM against BT474 cells and 11.4/6.8 nM against HER1/HER2. Compound **8** showed substantial suppression of tumor growth in NCI-N87 (94.8% inhibition) or SK-OV-3 xenograft (85.7% inhibition) models. Currently, selatinib is under phase I clinical trials (NCT01931943, clinicaltrials.gov) for its pharmacokinetic evaluation in advanced breast cancer subjects [51].

Ahmed *et al.* [52] carried out combined docking and molecular dynamic simulation studies on anilinoquinazoline-based anti-HER2 ligands. Molecular dynamic simulation studies showed that the compounds form stable complex with the binding site of the HER2 receptor. These compounds displayed various interactions in the hinge region and salt-bridge interactions with Asp863 and Lys753. Simulation studies revealed the importance of Vander-Waal's interactions for the ligand binding [52]. Ahmed *et al.* [53] carried out molecular dynamic simulations and binding energy calculations to find out the binding modes and the mechanisms of inhibition of *in silico* designed 4-anilinoquinazoline derivatives against HER1 and HER2. The most active compound showed H-bonding interactions with two amino acid residues, *i.e.*, Asp855 and Lys745 for HER1 and Asp863 and Lys753 for HER2. In addition, ligand showed H-bonding interaction with a conserved Met residue at the hinge region through the N1 atom of the quinazoline ring [53]. Sadek *et al.* [54] designed and synthesized anilinoquinazoline derivatives substituted at 4′ position of aniline by bulkyaryl-pyridinyl, arylpropenoyl and arylpyrazolyl moieties. Compound **9** (Fig. **4**), was found to be the most potent derivative

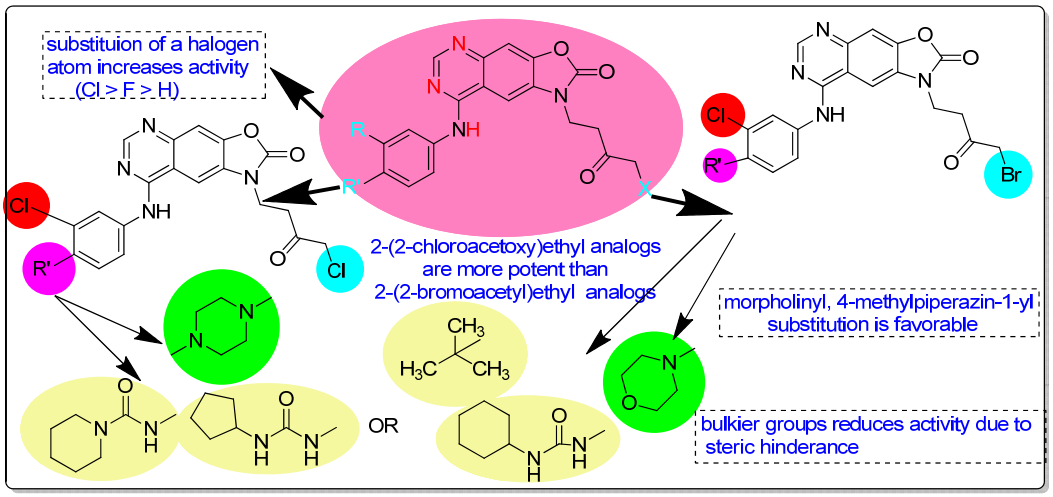

Fig. (6). SAR studies of anilinoquinazolines (compound **12**).

with IC_{50} values of 1.94 µM against HER1 and 1.04 µM against HER2. Besides, compound **9** also exhibited anti-proliferative activity against HER1 overexpressing MDA-MB-231 breast cancer cell lines [54].

Elkamhawy *et al.* [55] designed and synthesized 6-substituted 4-anilinoquinazolines as selective HER1/HER2 TKIs. Most of the compounds exhibited IC_{50} values in nanomolar range against HER1 and/or HER2 kinases. Computational studies indicated that four compounds showed favourable binding interactions at the ATP binding sites of both the kinases. In the series, compound **10** (Fig. **4**) displayed promising cytotoxicity with IC_{50} value of 1.82 µM against BT474 cell line. SAR analysis revealed that the replacement of fluorobenzyloxy moiety with phenoxy group reduced the antiproliferative activity [55]. Lyu *et al.* [56] have designed and synthesized a series of lapatinib derivatives by carrying out modifications of straight alkyl side chain of lapatinib into branched ones. These derivatives exhibited significant inhibition of HER1/HER2 as indicated by ELISA assay and western blot analysis. *In vitro* assay showed that these compounds possessed potent cytotoxicity against the HER1/HER2 overexpressing cancer cells. Amongst the synthesized lapatinib derivatives, compound, **11** (Fig. **4**) showed most potent antitumor activity, *in vivo,* with an IC_{50} value of 28.8 nM. It has been observed that the IC_{50} value of **11** was two folds higher than that of lapatinib (IC_{50}= 63.9 nM). Moreover, **11** was found to block the cell cycle progression of BT474 cells in the G_1 phase, causing tumor cell apoptosis. SAR studies showed that alkylamine substitution on the side-chain was beneficial for HER1/HER2 inhibitory and cytotoxic activity while bulkier substitution led to decreased activity. Further, stereochemical investigations of the compounds showed higher potency for (S)-isomers than for (R)-isomers [56]. Yin *et al.* [57] developed a series of oxazolo[4,5-g]quinazolin-2(1*H*)-one derivatives as HER1/HER2 TKIs displaying high activity and low toxicity. Compound **12** (Fig. **4**) showed the most potent HER1/HER2 kinase inhibition with IC_{50} value of 10/20 nM and displayed IC_{50} value of 0.47µM against SKBR3 cancer cell line.

In addition, compound **12** also exhibited anti-proliferative activity against human lung adenocarcinoma cell line (A549). In tumor xenograft models of lung cancer, compound **12** showed higher inhibition efficacy towards tumor growth as compared to lapatinib. *N1, N3* atoms in pyrimidine ring and a proton at the anilinic-N position were found to be crucial in this series of compounds. Introduction of electrophilic groups like 2-(2-chloroacetoxy)ethyl and 2-(2-bromoacetyl)ethyl at N-position of oxazolo ring was favourable for HER1/HER2 kinase inhibitory activity. The detailed SAR studies have been illustrated in Figs. (**6** and **7**) [57].

4.2. Pyrrolotriazene Analogs

Mastalerz *et al.* [58] synthesized and evaluated 5-methyl pyrrolotriazenes for HER1/HER2 kinase inhibitory activities. It was found that compound with *meta*-fluorobenzyl-indazolyl amino side chain displayed best inhibitory activity. Different C-5 substituted derivatives were investigated and through SAR studies, it was concluded that a basic group attached to C-5 of pyrrolotriazenes with a methylene ether linkage could show potent and selective dual inhibition of HER1/HER2 kinases. Compound **13** (Fig. **8**) was reported as the most potent in the series with IC_{50} values of 61/55 nM against HER1/HER2 respectively. It also displayed potent antiproliferative activity, enhanced oral exposure in rats and tumor xenograft models. Further, different C-4 substituted analogs of **13** were synthesized and SAR studies were summarized in Fig. (**9**) [58].

Mastalerz *et al.* [59] designed and synthesized C-5 substituted pyrrolotriazines and evaluated these as dual HER1/HER2 TK inhibitors. Most of the synthesized compounds showed HER1/HER2 inhibition in micromolar range. It was observed that the acid, ester and amide analogs of substituted piperidine ring resulted in less potency against HER1/HER2 overexpressing N87 gastric carcinoma cell line. Compound with a homopiperazine substituent at the C-5 position (**14**), (Fig. **8**) was the most potent derivative displaying significant kinase inhibition activity with IC_{50} values of 33/27 nM against HER1/HER2. In HER1/HER2 driven human tumor xenograft models compound **14** showed good oral efficacy (77% bioavailability) [59]. Mastalerz *et al.* [60] synthesized and evaluated a series of pyrrolotriazines with a 5-((4-aminopiperidin-1-yl)methyl) substitution as dual HER1/HER2 kinase inhibitors. The compound with amino-fluorobenzylindazole group at C-4 position (**15**), (Fig. **8**) was

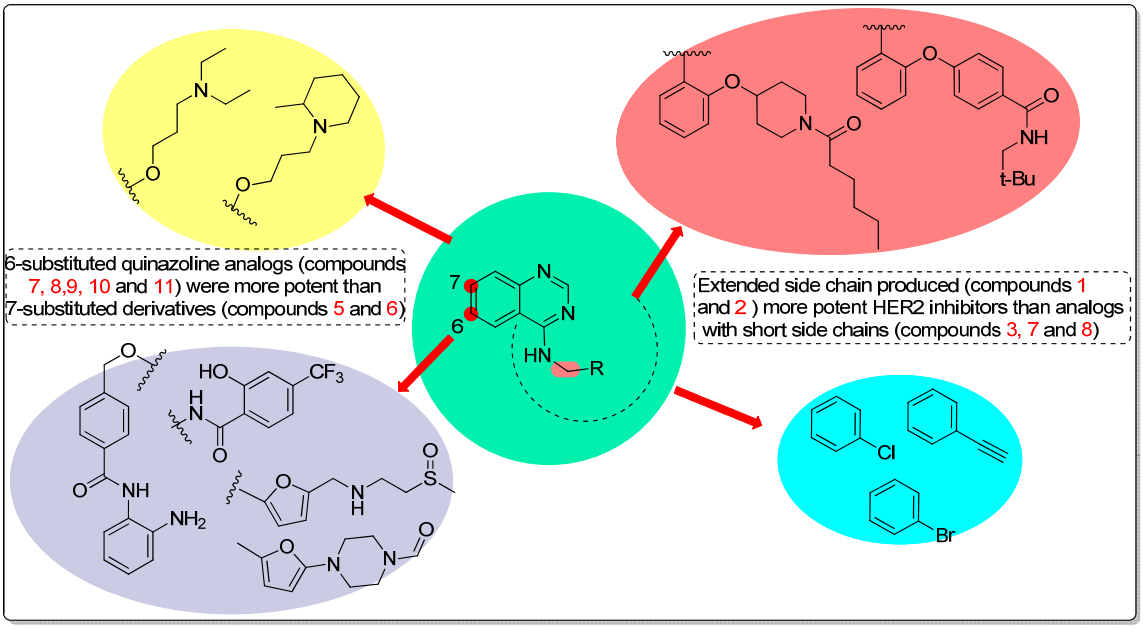

Fig. (7). SAR for various anilinoquinazoline derivatives acting as HER2 inhibitors.

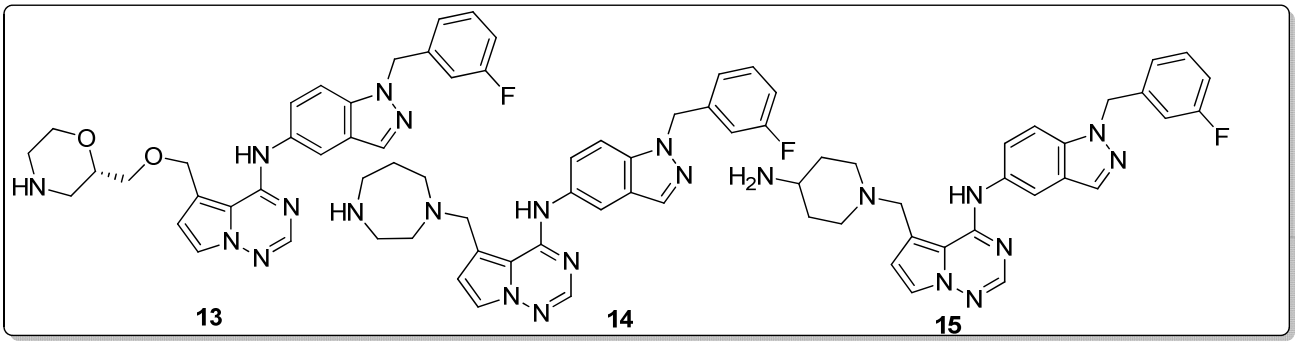

Fig. (8). Structures of pyrrolo-triazenes acting as HER2 inhibitors.

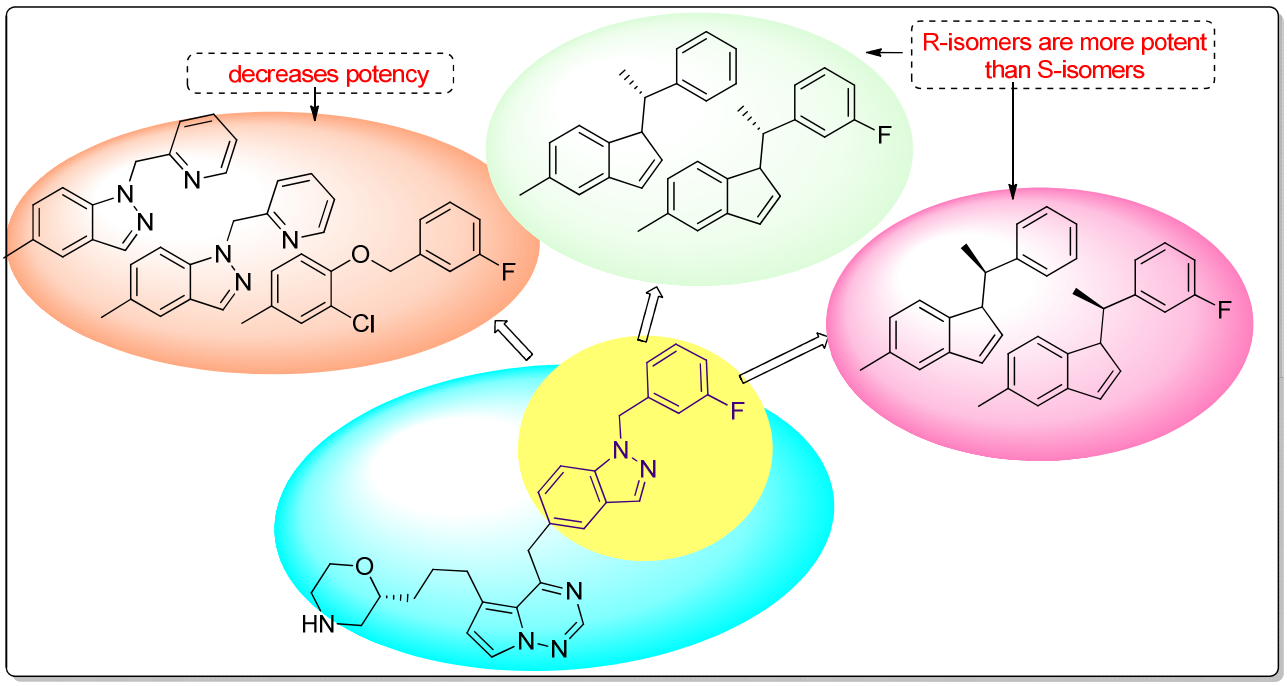

Fig. (9). SAR studies of pyrrolotriazenes (compound **13**).

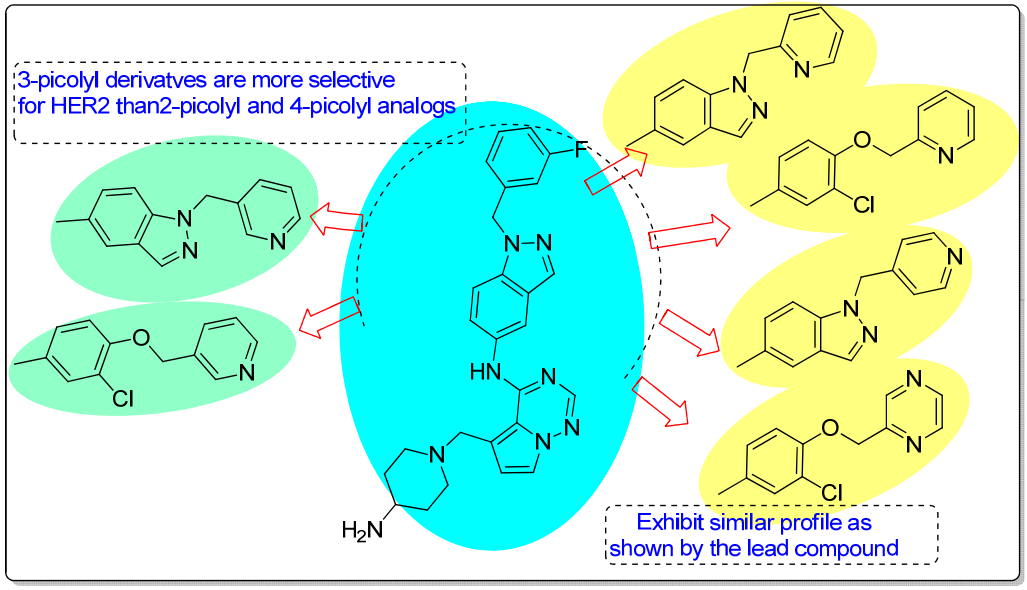

Fig. (10). SAR studies of pyrrolo-triazenes (compound **15**).

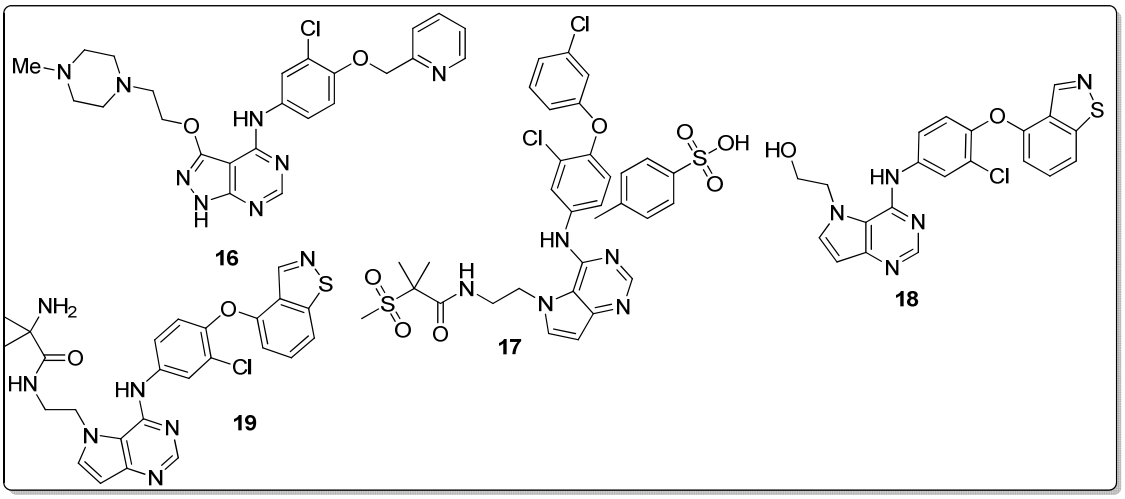

Fig. (11). Structures of pyrazolo- and pyrrolo-pyrimidines acting as HER2 inhibitors.

found to be the most active with IC_{50} values of 35 nM against N87 human gastric carcinoma cell line and 23 nM against HER2. SAR analysis of **15** has been described in Fig. (**10**) [60]. As clear from the IC_{50} data, the HER2 kinase inhibitory profile of C-5 aminopiperidinyl analog **15** is superior to that of previously synthesized pyrrolotriazene derivatives **13** and **14**.

4.3. Pyrazolo and Pyrrolo-pyrimidines

Ducray *et al.* [61] synthesized and evaluated 4-anilino-1*H*-pyrazolo[3,4-d]pyrimidines, *in vitro,* as HER1/HER2 kinase inhibitors. Compound **16** (Fig. **11**) was found to be the most potent among the synthesized derivatives exhibiting potent kinase inhibition activity with IC_{50} values of 5/1 nM against HER1/HER2, respectively. Pharmacokinetic studies of compound **16** showed good oral bioavailability in rat and dog and moderate half-life. This was because of moderate to high plasma clearance in rat (41 ml/min/kg) and dog (46 ml/min/kg). However, **16** displayed high intestinal absorption as it showed good permeability (9.5×10^{-6} cm s^{-1}) and limited efflux (efflux ratio 3.6) in MDCK-MDR1 assay. In addition, **16** showed insignificant activity for five isoforms of P450 enzyme with IC_{50}>10 µM. SAR studies are described in Fig. (**12**) [61].

Kawakita *et al.* [62] synthesized and screened various derivatives of TAK-285 as dual HER1/HER2 inhibitor. In order to improve the pharmacokinetic profile of TAK-285, chemical modifications were performed at the *N*-5 side chain of the pyrrolo[3,2-*d*]pyrimidine core and final products were converted into their salts. Compound **17** (Fig. **11**) as a tosylate salt exhibited potent HER1/HER2 kinase inhibitory activity with IC_{50} values of 11/11 nM, respectively and cellular growth inhibitory activity with GI_{50} value of 56 nM against BT474 cell line. The salt form of **17** showed higher solubility and good pharmacokinetic properties than the free form (Fig. **13**).

Kawakita *et al.* [63] also explored bicyclic fused ring containing pyrrolo[3,2-d]pyrimidines derivatives as HER1/HER2 inhibitors. A total of 22 compounds were screened and 1,2-benzisothiazole derivatives (**18**), (Fig. **11**) showed potent HER1/HER2 inhibition with IC_{50} values of 2.1 nM and 4.1 nM respectively. Compound 18 was also

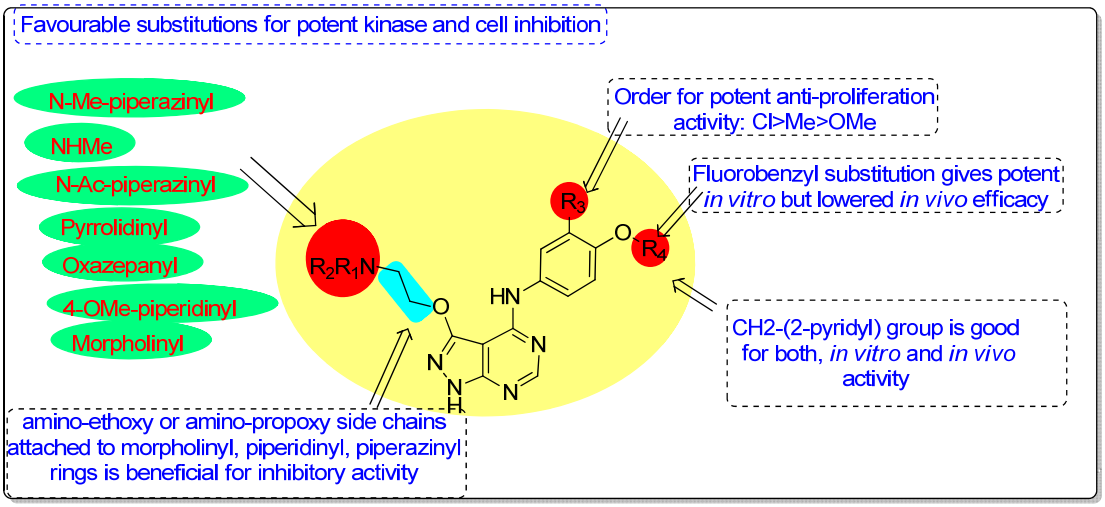

Fig. (12). SAR studies of pyrazolo-pyrimidines (compound **16**).

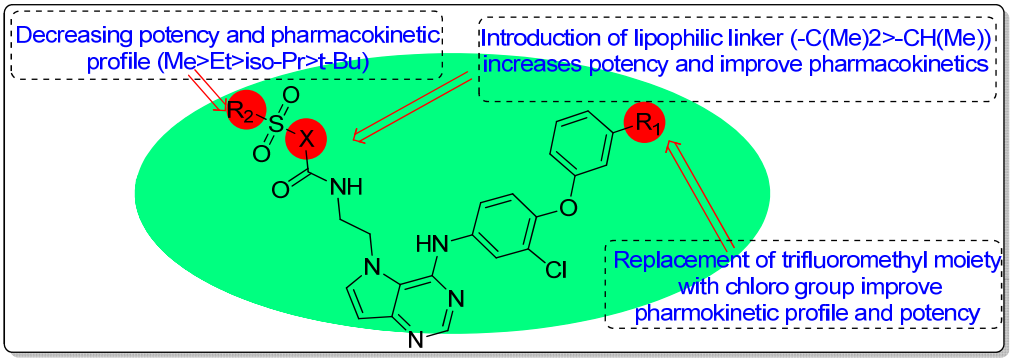

Fig. (13). SAR studies of pyrrolo-pyrimidines (compound **17**).

effective against BT474 cancer cell lines with IC_{50} value of 3.6 nM. Docking studies revealed that **18** showed H-bonding interactions with Thr798 and Ser783 of HER2 kinase. Further optimization of *N*-5 substituents on the pyrrolo[3,2-*d*]pyrimidine core was done to improve the *in vitro* oxidative metabolic stability in human hepatic microsomes. This resulted in the discovery of a preclinical candidate **19** (Fig. **11**) with reduced cytochrome P450 inhibitory activity and enhanced metabolic stability. Compound **19** showed IC_{50} value of 2.5/0.98 nM against HER1/HER2 and GI_{50} value of 2.0 nM against BT474 cancer cell lines [63]. The HER2 inhibitory profile of benzisothiazole derivatives of pyrrolo-pyrimidine series was better than the previously designed pyrrolo-pyrimidine derivatives.

4.4. Thieno-pyrimidines

Hubbard *et al.* [64] synthesized and evaluated a series of pyrrolidinyl-acetylenic thieno[3,2-d]pyrimidines as selective HER1/HER2 inhibitors. In the series, compound **20** (Fig. **14**) displayed IC_{50} values of 32/43 nM against HER1/HER2 with good bioavailability (Dose-normalised area under plasma-drug concentration curve, DNAUC = 61 ng h/ml/mg/kg). SAR studies showed that any substitution at the pyrrolidinic nitrogen was detrimental for the activity. Carbamoyl derivatives at the C-4 position showed good enzyme and cell growth inhibitory activities [64]. Waterson *et al.* [65] synthesized thieno[3,2-d]pyrimidines and evaluated them as effective dual inhibitors of HER1 and HER2 kinases. SAR studies for the synthesized series of compounds have been shown in Fig. (**15**). Compounds **21** and **22** (Fig. **14**) were found to be the potent and HER2-selective kinase inhibitors with IC_{50} values of 50/30 nM and 80/50 nM, respectively against HER1/HER2. Compounds **21** and **22** also displayed inhibitory activity against BT474 cancer cell line with IC_{50} values of 60 nM and 30 nM, respectively [65].

Rheault *et al.* [66] synthesized two series of selective dual HER1/HER2 kinase inhibitors derived from thieno[3,2-d]pyrimidine scaffold. Most of these compounds showed good *in vitro* anti-proliferative activity against human tumor cells. In these series, compound **23** (Fig. **14**) was the most potent and selective HER2 inhibitor with IC_{50} values of 12/6 nM against HER1/HER2 respectively. The compound was 10 to 1000-fold selective for HER1 and HER2 over a variety of other kinases. SAR analysis indicated that grafting of unsubstituted heterocycles such as furan, pyrrole and thiophene rings onto thieno-pyrimidine ring reduces kinase inhibition and anti-proliferative activities. While, H-bond donor group on the side chain attached to thieno-pyrimidines enhance potency [66]. An increased HER2 inhibition and selectivity was observed for this series as compared to the simple thieno-pyrimidine derivatives.

4.5. N-aryl Pyrimidines

Xu *et al.* [67] reported a series of *N,N*-disubstituted hydrazones as selective TK inhibitors and antiproliferative agents. In the series, compound **24** (Fig. **16**) was found to be

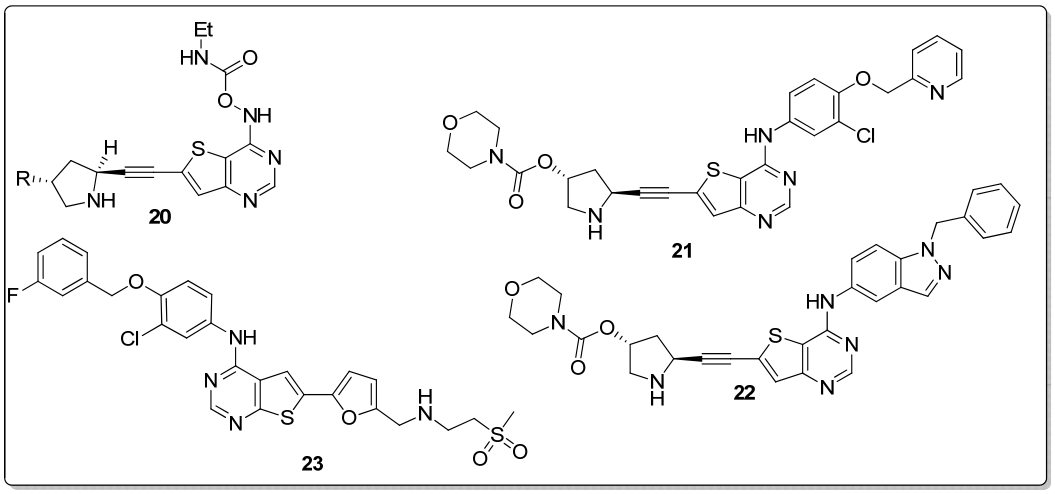

Fig. (14). Structures of thieno-pyrimidines acting as HER2 inhibitors.

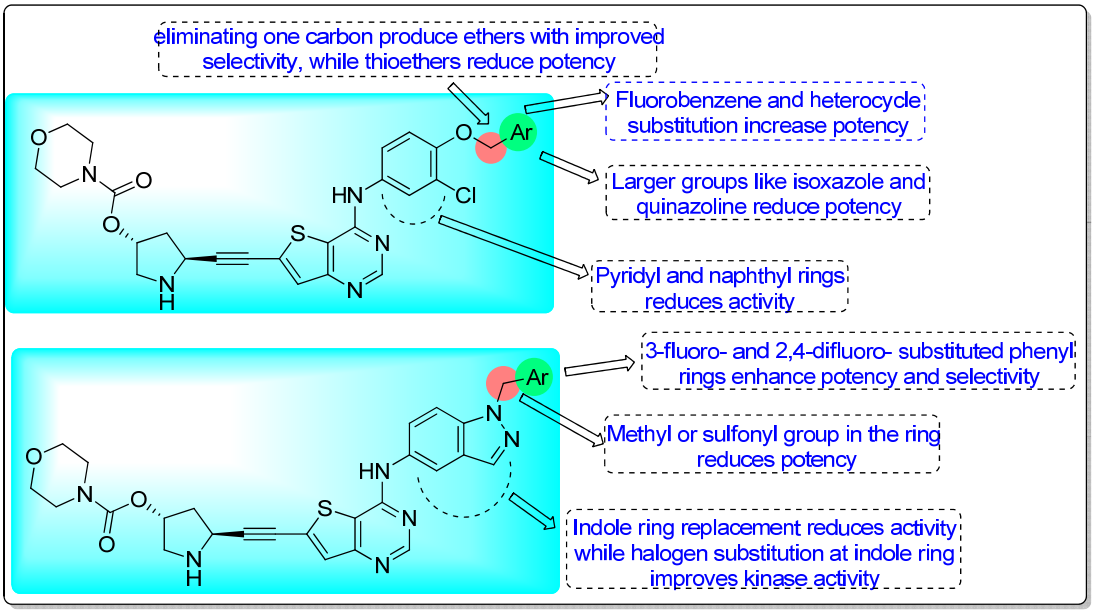

Fig. (15). SAR studies of thieno-pyrimidines (compounds **21-22**).

the most potent against BT474 and SKBR3 breast cancer cell lines with IC_{50} values of 14 nM and 58 nM respectively. In addition, **24** inhibited growth factor-induced receptor phosphorylation in SKBR3 cells (IC_{50}= 54 nM) and inhibited HER1/HER2 TK with IC_{50} values of 9/8 nM. SAR studies demonstrated that dimethylated amino analog **(24)** and cyclic rings such as morpholinyl, piperidinyl, piperazinyl, pyrrolidinyl were good for the activity. Mono-substituted amino analogs were less active than disubstituted and cyclised amino analogs [67].

Hughes *et al.* [68] conducted SAR studies on *N*-aryl-4,6-pyrimidine diamines. In this series, oxadiazole analog **25** (Fig. **16**), exhibited potent growth inhibitory activity against BT474 (IC_{50}= 312 nM), and SKBR3 (IC_{50}= 325 nM) cancer cell lines. Interestingly, **25** did not inhibit the growth of non-HER2 dependent HeLa cell lines (IC_{50}> 100μM). In addition, **25** exhibited selectivity for HER family kinases and showed minimal activity against Aurora-A, CDK1, and VEGF-R2. SAR studies indicated that oxadiazole and hydrazide analogs were potent inhibitors. Replacement of oxadiazole ring with other groups followed this order for activity: CHO<COOH=CN<COOMe. The bulkier ester and amide analogs displayed minimal efficacy [68]. Xu *et al.* [69] synthesized and evaluated 4-amino-6-arylaminopyrimidine-5-carbaldehyde oxime derivatives (SAR shown in Fig. **17**). These compounds were potent inhibitors of both HER1 and HER2 TKs and showed antiproliferative activity against SKBR3 and BT474 cancer cell lines. Compound **26** (Fig. **16**) was found to be the most potent with IC_{50} values of 8/12 nM against HER1/HER2 kinase and significantly inhibited SKBR3 (IC_{50}=0.26 μM) and BT474 (IC_{50}=0.25 μM) cancer cell lines [69].

Suzuki *et al.* [70] synthesized and reported the HER1/HER2 kinase inhibitory activity of 5-alkenyl or 5-alkynyl-4-anilinopyrimidines against BT474 cell line. In these series, compound **27** (Fig. **16**) was found to be the most potent with IC_{50} values of 62 nM and 50 nM against HER2 and BT474 cancer cell lines, respectively. Compound **27** displayed significant antitumor potency in a mouse xenograft model. Further, compound **27** displayed better

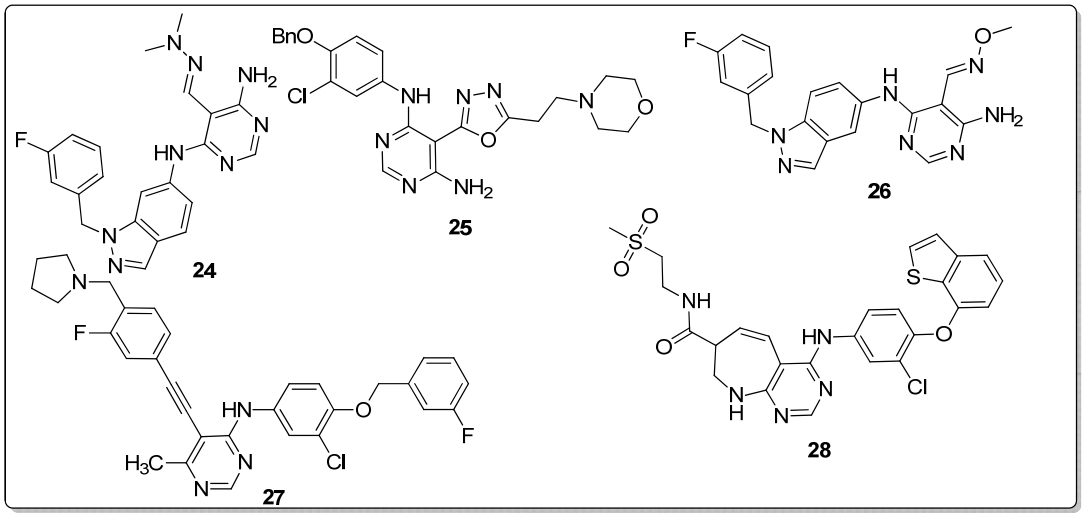

Fig. (16). Structures of *N*-aryl pyrimidines acting as HER1/HER2 inhibitors.

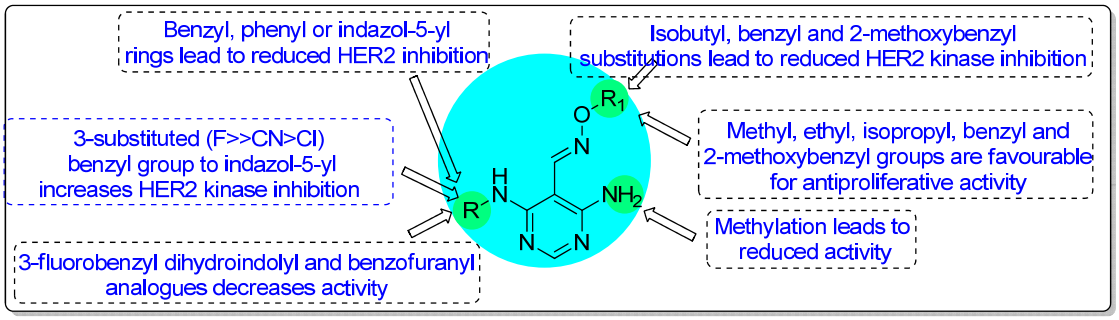

Fig. (17). SAR studies of *N*-aryl pyrimidines (compound **26**).

aqueous solubility as compared to lapatinib and showed good stability in human liver microsomes. 5-Alkynyl moiety played critical role in the activity of the compounds. It was observed that alkynylpyrimidines were more potent than the corresponding alkenylpyrimidines. Presence of unsubstituted phenyl ring at the acetylinic position resulted in decreased activity, while the substituted benzyl analogs were more potent. The attachment of alkylamines to benzyl ring led to significant increase in the activity as the terminal amine group interacts with Asp776 of HER1 and Asp808 of HER2. Introduction of fluorine at the *meta* position of benzyl ring (compound **27**) led to enhanced inhibition of cellular proliferation [70]. Kawakita *et al.* [71] synthesized 7,6-fused bicyclic pyrimido[4,5-b]azepines *via* an intramolecular Claisen type condensation. In this series, compound **28** (Fig. **16**) displayed potent HER1/HER2 kinase inhibition activity with IC_{50} values of 36/24 nM, respectively and inhibited BT474 cancer cell line growth with GI_{50} value of 18 nM. SAR studies showed that 1-benzothiophene derivatives were more active than the 3-trifluoromethylphenoxy derivatives [71].

4.6. Miscellaneous Analogs

Cheng *et al.* [72] designed and synthesized 4-Aryl-5-cyano-2*H*-1,2,3-triazoles with different types of substitutions at the 4-phenyl position. Compound **29** (Fig. **18**) was found to be the most potent amongst the synthesized derivatives with IC_{50} values of 6.6 µM for HER2 TK inhibition and 30.9 µM against breast cancer cells (MDA-MB-453). Compound **29** showed significant binding interaction with Cys805 in the ATP binding site of HER2 kinase domain. From the SAR studies, it was concluded that a 4-phenyl group at the triazole with a methoxy, dimethoxy or trimethoxy substituent is detrimental for the activity while a halogen or a phenoxy group is favourable for the HER2 activity [72]. Gundla *et al.* [73] employed virtual screening models to discover a set of structurally diverse compounds with growth inhibitory activity against HER2-overexpressing SKBR3 breast cancer cell line. From the 3D database of 350000 small-molecules, a total of 531 potential hits were retrieved using consensus models. Amongst the 531 potential hits, 57 compounds were tested against SKBR3 cell line on the basis of structural novelty and drug like properties. In this series, seven compounds inhibited growth of SKBR3 cell lines with IC_{50} values less than 10 µM. Compounds **30** and **31** (Fig. **18**) were found most potent with IC_{50} values of 2 µM each. SAR studies of these analogs have been described in Fig. (**19**) [73]. Ding *et al.* [74] synthesized and evaluated salicylanilide derivatives as HER1 and HER2 TK inhibitors. A number of compounds were evaluated and compound **32** (Fig. **18**) was found to exhibit dual inhibitory activities against HER1/HER2 (IC_{50}= 1.654/7.134 µM). Compound **32** could be taken as a lead compound for further optimization to develop potent inhibitors [74].

Wang *et al.* [75] designed and synthesized a series of thiazolyl-pyrazoline derivatives containing benzodioxole ring as HER2 kinase inhibitors. The most potent compound **33**

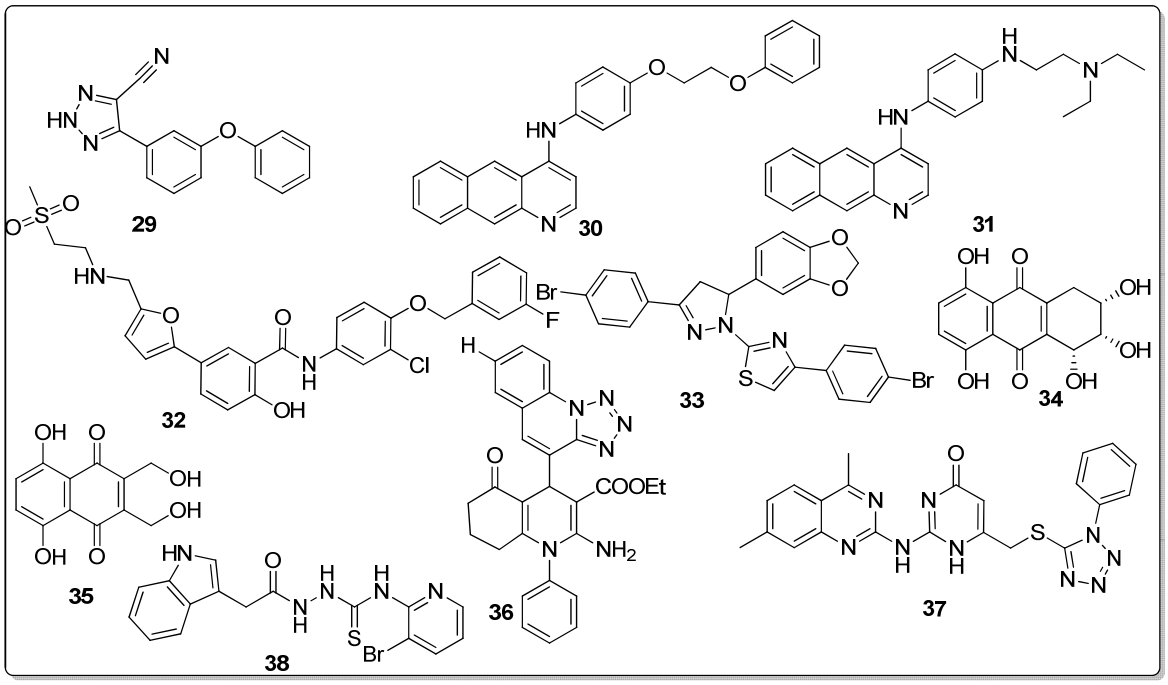

Fig. (18). Miscellaneous scaffolds acting as HER2 inhibitors.

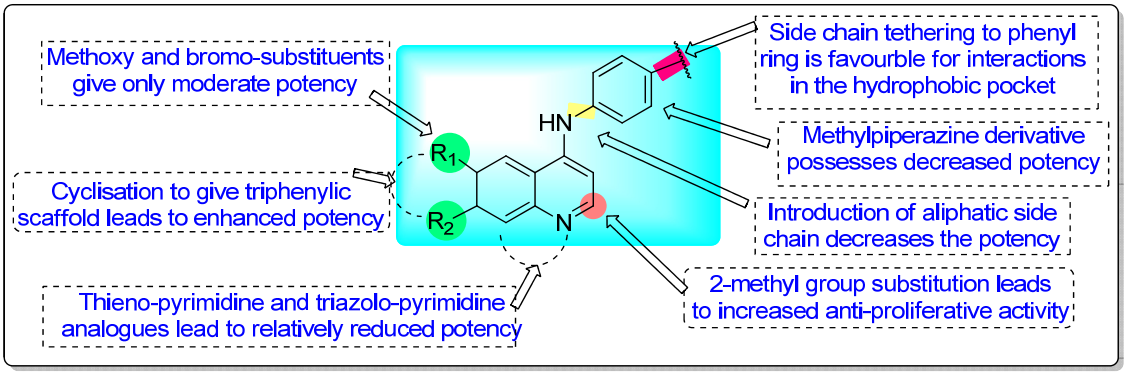

Fig. (19). SAR studies of compounds **30-31**.

(Fig. **18**) displayed HER2 kinase inhibition activity with an IC_{50} value of 0.18 µM. The compound **33** was also evaluated, *in vitro*, for antiproliferative activities against MCF-7 and B16-F10 cell lines and displayed IC_{50} values of 0.09 µM and 0.12 µM, respectively. Docking simulation studies showed

two hydrogen bonding interactions with Thr347 and Arg394 of HER2 kinase. Different substituents at the *para*-position of ring A followed the activity order of Br > Cl > F, Br > Me >OMe [75]. Sridhar *et al.* [76] reported compounds **34** and **35** (Fig. **18**) displaying inhibition of breast tumor cells expressing HER2 with IC_{50} values of 0.87 µM and 0.82 µM, respectively. These compounds inhibited trastuzumab resistant HER2 oncogenic isoform, HER2Δ16 with IC_{50} values of 0.91 µM and 0.93 µM, respectively. It has been found that these compounds inhibit auto-phosphorylation of Y1248 and Y1068 residues of HER2 and HER1, respectively [76]. Sangani *et al.* [77] designed and synthesized biquinoline–pyridine hybrids and evaluated their HER1/HER2 kinase inhibition potential. Almost, all the compounds showed effective anti-proliferation activity against A549 and HepG2 carcinoma cell lines. Compound **36** (Fig. **18**) was found to be the most potent HER1/HER2 kinase inhibitor showing IC_{50} values of 0.09/0.2 µM. SAR studies revealed that when hydrogen atom (shown on **36**) was replaced with substituents like -OCH_3, -CH_3 and Cl the kinase activity decreases and follows the order of OCH_3>CH_3>Cl [77]. Elseginy *et al.* [78] identified a series of compounds *via* virtual screening that showed HER1/HER2 kinase inhibitory activity and potent anticancer activity against both the BT474 and SKBR3 cancer cell lines. In the series, Compound **37** (Fig. **18**) was found to be the most active against SKBR3 (IC_{50}=1.03 µM) and BT474 (IC_{50}=0.3139 µM) cell lines, and showed more than 80% inhibition against HER1/HER2 kinases. Compound **37** displayed H-bond interaction with Met801 and hydrophobic interactions with Leu852, Leu762, Phe1004, Thr798, Thr862 and Leu785 in the HER2 kinase domain. Another series was further designed and synthesized by merging the structural features of all the hit compounds. Compound **38** was the most potent in the series displaying inhibitory activity with IC_{50} value of 0.01 µM against SKBR3 cancer cell line and 0.055 µM against BT474 cancer cell line, besides showing HER1/HER2 kinase inhibitory activity with IC_{50} values of 8.49/12.37 µM respectively [78].

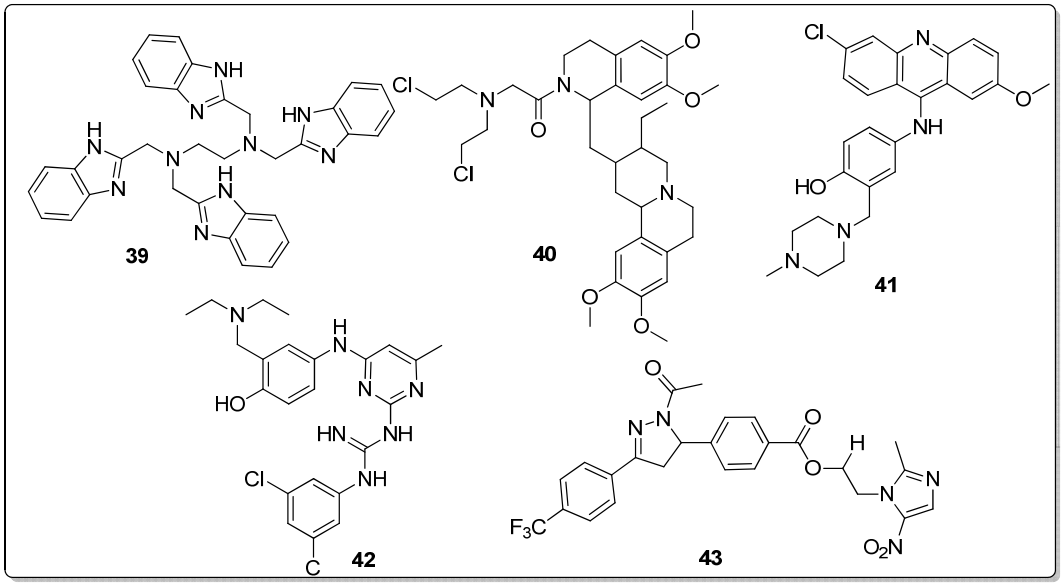

Fig. (20). Miscellaneous scaffolds acting as HER2 inhibitors (Contd.).

Zalloum *et al.* [79] identified HER2 inhibitors using pharmacophore modeling and QSAR analysis, which were further evaluated for inhibitory activities against HER2-overexpressing SKOV3 ovarian cancer cell lines and MCF-7 cancer cell line. Out of 80 inhibitors identified by *in silico* mining, four compounds (**39, 40, 41**, and **42** shown in Fig. **20**) were found to inhibit the growth of SKOV3 cells with IC_{50} values less than 5 μM and showed no activity against MCF-7 cancer cell lines [79]. Tao *et al.* [80] synthesized pyrazole-nitroimidazole derivatives and evaluated these for HER1/HER2 TK inhibitory activities and for their antiproliferative potential against MCF-7, Hela, HepG2 and B16-F10 cancer cell lines. Compound **43** was the most potent HER1/HER2 tyrosine kinase inhibitor (Fig. **20**) with IC_{50} values of 0.26/0.51 μM. Compound **43** also exhibited antiproliferative activity against human cervical cancer cell line (Hela) with an IC_{50} value of 0.13 μM. It has been observed that there was increase in the antiproliferative activity against Hela or HepG2 cell lines in the presence of electron-withdrawing groups [80].

5. DRUGS UNDER CLINICAL INVESTIGATION TARGETING HER2 TK

Different HER2 TK inhibitors presently under clinical evaluation for the treatment of breast cancer have been shown in Figs. (**21** and **22**).

5.1. Lapatinib (GW572016)

Lapatinib is a reversible oral, dual TK antagonist of HER1 and HER2. It prevents the phosphorylation of their substrates and downstream signaling [81-83]. Lapatinib displayed IC_{50} values of 10.2 nM and 8.9 nM against HER1 and HER2 TKs, respectively [84]. It showed activity against trastuzumab-resistant cancer cell lines. Lapatinib in combination with capecitabine was approved by FDA in March, 2007 for the treatment of metastatic breast cancer in patients who have received trastuzumab, paclitaxel and anthracycline drug regimens [85]. The combination is found better than the trastuzumab therapy because of its activity against p95HER2 which lacks the trastuzumab-binding domain [35]. Lapatinib enhances sensitivity towards endocrine treatment for the HR-positive patients [86]. Lapatinib in combination with letrozole, was approved by FDA in February, 2010 as a first-line therapeutic option for the treatment of post-menopausal metastatic breast cancer patients co-expressing HR and HER2 (www.accessdata.fda.gov) [87]. However, HER2 positive breast cancer patients develop resistance towards lapatinib monotherapy [88]. The resistance for lapatinib is attributed to the HER3 transphosphorylation and activation of PI3K-Akt pathway due to negative feedback signaling loop [89] and enhanced ER signaling [90]. Lapatinib in combination with panobinostat and capecitabine (NCT00632489, clinicaltrials.gov) [91] and with sirolimus/metformin (NCT0108 7983, clinicaltrials.gov) [92] has completed phase I clinical trials for the treatment of HER2 positive metastatic breast cancer. Lapatinib along with cabazitaxel (NCT01934894, clinicaltrials.gov) has completed phase II clinical trial for treating breast cancer patients who are HER2 positive and suffering from intracranial metastases [93]. Lapatinib is currently undergoing phase II clinical trials (NCT00820872, NCT01827163; clinicaltrials.gov) [94-95] and phase III trial (NCT00553358, clinicaltrials.gov) [96] in various combinations of trastuzumab and other chemotherapeutic agents for the treatment of HER2 positive early stage breast cancer and as neoadjuvant therapy for the HER2-positive breast cancer. Apart from this, lapatinib is undergoing various other clinical studies for the treatment of breast cancer and other solid tumors (www.clinicaltrials.gov).

5.2. Afatinib

Oral afatinib (BIBW 2992 / Giotrif / Gilotrif; Boehringer Ingelheim) is an approved first-line treatment for HER1-positive metastatic non-small cell lung carcinoma (NSCLC) patients, possessing HER1 exon 19 deletions or exon 21 (L858R) substitution mutations (www.accessdata.fda.gov). Afatinib is orally bioavailable pan-HER inhibitor targeting HER1, HER2 and HER4 *via* irreversible covalent binding interactions. It also inhibits HER3 transphosphorylation [97]. Afatinib exhibits covalent interactions with Cys797 of

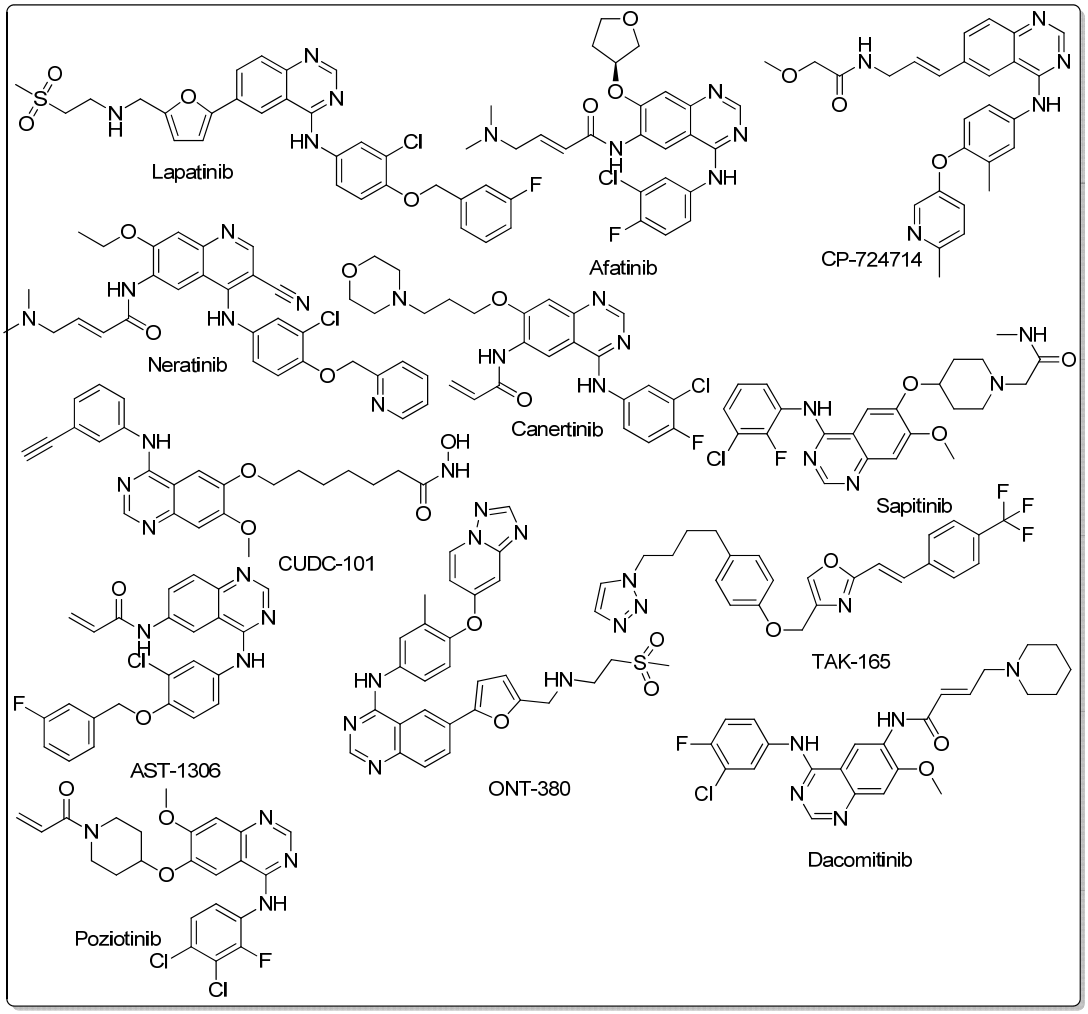

Fig. (21). Structures of HER2 inhibitors undergoing clinical trials.

HER1, Cys805 in HER2 and Cys803 in HER4 [98, 99]. It showed half-maximal inhibitory concentration values of 0.5 nM, 14 nM and 1 nM against HER1, HER2 and HER4, respectively [98-99]. The drug retains *in vivo* and *in vitro* anticancer activity in tumors that acquire resistance towards reversible HER1 inhibitors due to T790M mutation [100-106]. Although, afatinib is an effective first-line treatment for NSCLC but the drug is still under clinical evaluation for the treatment of HER2 positive breast cancer (clinicaltrials.gov). Afatinib and/or vinolerobine has completed phase II clinical trials (NCT01441596 and NCT01325428, clinicaltrials.gov) for the treatment of HER2 overexpressing metastatic breast cancer patients with extracranial brain metastases and HER2 overexpressing inflammatory breast cancer [107-108]. Afatinib has successfully completed phase II trial (NCT 00431067, clinicaltrials.gov) for the treatment of trastuzumab-refractory HER2 positive metastatic breast cancer. The toxicity profile was also manageable however, frequent diarrhea was observed with afatinib monotherapy [109]. Afatinib has been found to be quite effective over trastuzumab and lapatinib (NCT00826277, clinicaltrials.gov) in the neo adjuvant treatment of HER2 positive breast cancer [110]. Afatinib alone or in combination with vinolerobine has undergone phase II clinical evaluation (NCT01271725, clinicaltrials.gov) for treating HER2 positive breast cancer patients who were not responding to HER2 targeted therapy in adjuvant/ neoadjuvant settings [111]. Recently, Cortes *et al.* reported that afatinib containing treatment were not effective against HER2 positive breast cancer and was less tolerated and frequent adverse events were observed [112]. In combination with trastuzumab, the drug has completed phase I clinical evaluation (NCT01649271, clinicaltrials.gov) for treating HER2 overexpressing breast cancer.

5.3. Sapitinib (AZD8931)

Sapitinib (AZD8931) is a reversible equipotent inhibitor of HER1, HER2 and HER3-mediated signaling dynamics displaying IC_{50} values of 4 nM, 3 nM and 4 nM respectively [113, 114]. Methyl acetamide chain of sapitinib contributes to the increased HER2 activity. It exhibited potent tumor growth inhibition in various xenograft models either expressing HER1 or co-expressing HER1 and HER2 [114]. Sapitinib in combination with paclitaxel has successfully completed phase II clinical trials (NCT00900627, clinicaltrials.gov) for treating female subjects suffering from advanced breast cancer with low HER2 overexpression [115].

5.4. Neratinib (HKI-272)

Neratinib is an oral, irreversible inhibitor of HER1/HER2 with IC_{50} values of 92/59 nM [116-119]. Neratinib has

shown improved invasive disease-free survival in phase III clinical evaluation (NCT00878709, clinicaltrials.gov) in HER2 positive early breast cancer patients who have previously obtained adjuvant trastuzumab therapy [120]. The drug in combination with capecitabine and vinorelbine, (NCT00741260 and NCT00706030, clinicaltrials.gov) respectively has shown promising antitumor activity in HER2 positive metastatic breast cancer patients and the toxicity profile was also manageable [121, 122]. Neratinib as monotherapeutic agent has shown good clinical activity and acceptable tolerability (NCT00777101, clinicaltrials.gov) in HER2 positive recurrent or metastatic breast cancer. Moreover, it can also be used as an alternative therapy for patients who do not respond to therapeutic combination of lapatinib and capecitabine [123]. The combination of neratinib and paclitaxel in phase II study (NCT00915018, clinicaltrials.gov) has shown similar results when compared with trastuzumab-paclitaxel combination in term of progression free survival. However, neratinib and paclitaxel combination has been found more effective in reducing CNS progression associated with HER2 positive breast cancer [124]. Neratinib as single agent has shown marked improvement in a young woman suffering metastatic breast cancer and carrying HER2 LSS5 mutation in HER2 tyrosine kinase domain [125].

5.5. CP-724, 714

CP-724,714 is a reversible, potent and selective oral HER2 TK inhibitor exhibiting IC_{50} value of 10 nM. It induced cell-cycle arrest at G_1 phase in BT474 carcinoma cell lines. Treatment with CP-724,714 decreases HER2 receptor phosphorylation in *ex vivo* studies [126]. It was found to foster cell apoptosis and reduction in downstream receptor TK signaling. It caused tumor-regression in HER2 overexpressing tumor cell lines [126]. The drug inhibited hepatic efflux transporters that caused hepatic accumulation of drug and bile constituents leading to hepatocellular injury and hepatobiliary cholestasis [127]. Since then the clinical development of drug has been terminated.

5.6. Canertinib (CI-1033)

Canertinib (CI-1033) is an oral, irreversible pan-HER TK inhibitor, obstructing signaling from all members of the EGFR family *i.e.*, HER1, HER2, HER3 and HER4 [128]. It showed IC_{50} values of 1.5/9.0 nM against HER1/HER2 [129]. In a randomized phase II trial, the drug exhibited no significant clinical activity as a monotherapeutic agent in heavily pre-treated metastatic breast cancer patients. However, antitumor activity with enhanced overall survival rate was observed in one arm of HER2 positive patients at a drug dosage of 50 mg. Toxicity was observed at higher doses [130].

5.7. CUDC-101

CUDC-101 is an irreversible, multi-targeted inhibitor of HDAC, HER1, and HER2 with IC_{50} values of 4.4 nM, 2.4 nM, and 15.7 nM, respectively [131]. In various xenograft models, the drug has shown tumor regression. In addition, CUDC-101 also exhibited inhibition of other alternate signaling pathways such as Akt, HER3, MET and hence, conquering the restraints of conventional HER1/HER2 inhibitors [132]. It has successfully completed phase I clinical trial (NCT01171924, clinicaltrials.gov) in advanced head and neck, gastric, breast, liver and non-small cell lung cancer. The drug is well-tolerated and exhibit good antitumor activity [133].

5.8. AST-1306

AST-1306 is an oral and irreversible inhibitor of HER1/HER2 with IC_{50} values of 0.5/3.0 nM. It binds through covalent interaction with Cys797 and Cys805 in the catalytic domains of HER1 and HER2, respectively. HER2 overexpressing cell lines, tumor xenografts and breast cancer mouse models showed more sensitivity towards the drug as compared to HER1 overexpressing cell lines [134]. AST-1306 completed phase I open-label dose-escalation study to determine its safety and tolerability, pharmacokinetics and preliminary anti-tumor effects which indicated rapid absorbance of the drug with moderate to high clearance. The maximum tolerated dose of the drug was found to be 1000 mg and it was further recommended for additional phase II trials [135].

5.9. Dacomitinib (PF00299804)

Dacomitinib (PF00299804) is an oral irreversible pan-HER inhibitor of HER1, HER2, and HER4 with IC_{50} values of 6.0 nM, 45.7 nM, and 73.7 nM, respectively. The drug inhibited wild-type HER2 and the gefitinib-resistant oncogenic HER2 mutation in lung cancers [136]. Dacomitinib exhibited significant antitumor activity in tumor xenograft models expressing and/or overexpressing HER family members or having mutations in HER1 [137]. Dacomitinib caused G_0-G_1 arrest and apoptosis induction in order to exert its antiproliferative activity. An escalating growth inhibitory activity was observed for HER2-amplified cell lines which was maintained by the drug in the corresponding cell lines showing resistance against trastuzumab and lapatinib [138]. Dacomitinib, as monotherapeutic agent has completed an open-label phase II trial (NCT01152853, clinicaltrials.gov) in HER2 positive advanced gastric cancer patients who have prior failed at least one chemotherapy regimen [139].

5.10. TAK-165

TAK-165 potently inhibits HER2 TK with an IC_{50} value of 6.0 nM. *In vitro* and *in vivo* studies have confirmed the anti-proliferative effects of TAK-165 against HER2-overexpressing cancers *viz*., bladder, kidney and androgen-independent prostate cancer [140]. The drug has completed phase I clinical trial (NCT00034281, clinicaltrials.gov) in patients with HER2 tumor expression.

5.11. ONT-380 (ARRY-380)

ONT-380 (ARRY-380) is a small-molecular TK inhibitor of HER2 with an IC_{50} value of 8 nM. It was found to be 500 times more selective for HER2 as compared to HER1 [141]. The drug in combination with trastuzumab and ado-trastuzumab is undergoing phase I clinical trials (NCT 01921335 and NCT01983501, respectively; clinicaltrials.gov) for the treatment for HER2 positive advanced breast cancer [141, 142].

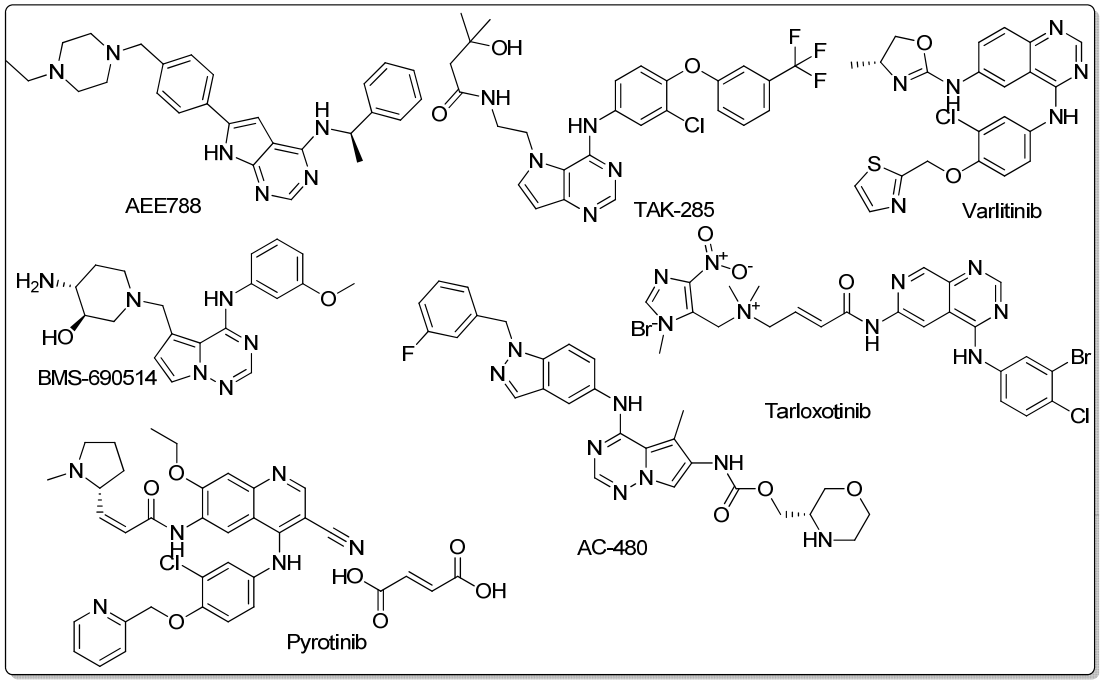

Fig. (22). Structures of HER2 inhibitors under clinical trials (Contd.).

5.12. Poziotinib (HM781-36B; NOV120101)

Poziotinib is an irreversible pan-HER inhibitor with IC_{50} values of 3 nM, 5 nM and 23 nM for HER1, HER2, and HER4, respectively. It has shown anti-proliferative effects against both HER1 and HER2 overexpressing cell-lines and tumor xenografts. Besides, it exhibited an IC_{50} value of 1.0 nM against HER2 overexpressing SKBR3 cell lines [143]. Poziotonib stimulated cell cycle arrest at G_1-phase and apoptosis, and reduces the levels of HER family and downstream signaling molecules [144]. It displayed potent *in vitro* and *in vivo* antitumor activity against HER2 amplified gastric cancer cells (SNU216 and N87). In combination with chemotherapeutic agents, synergistic effects of poziotinib were seen in both HER2 amplified and HER2 non-amplified gastric cancer cells [145]. In metastatic HER2 positive breast cancer patients**,** drug acclaims promising clinical activity [146]. At present, poziotinib is undergoing phase II evaluation (NCT02418689, clinicaltrials.gov) in patients with HER2-positive metastatic breast cancer who have received at least two prior HER2-directed regimens [147].

5.13. Varlitinib (ARRY-334543; ASLAN001)

Varlitinib is a potent, reversible, tyrosine kinase inhibitor of HER1, HER2 and HER4 showing IC_{50} values of 7 nM, 2 nM and 0.195 nM, respectively. It has completed phase II study (NCT01614522, clinicaltrials.gov) in patients with recurrent/metastatic gastric cancer whose tumors are either HER2 amplified or co-expressing HER1 and HER2 [148].

5.14. Pyrotinib

Pyrotinib is an irreversible TK inhibitor with IC_{50} values of 5.6/8.1 nM against HER1/HER2 [149]. The drug is undergoing phase I clinical evaluation among HER2 positive advanced breast cancer patients (NCT01937689, clinicaltrials.gov). Moreover, promising clinical activity of pyrotinib has been reported in patients with HER2 positive metastatic breast cancer who had previously undergone treatment with anthracyclines, taxanes, and trastuzumab [149].

5.15. BMS-690514

BMS-690514 inhibited the HER1, HER2 and vascular epidermal growth factor receptor 2 (VEGFR2) with IC_{50} values of 5 nM, 20 nM and 50 nM, respectively. Its efficacious anti-tumor profile has been seen in a number of tumor xenograft models including BT474, N87, L2987, Sal2, and GEO, all being dependent on HER1 and/or HER2 signaling. It has displayed superior efficacy profile than HER1 or pan-HER inhibitors such as gefitinib or BMS-599626 that may be attributed to its anti-angiogenic properties [150]. The drug in combination with letrozole, has completed phase I clinical trials (NCT01068704, clinicaltrials.gov) for the treatment of metastatic breast cancer.

5.16. Tarloxotinib (TH-4000)

Tarloxotinib bromide is a prodrug that is activated under hypoxic conditions to release an irreversible HER1/HER2 inhibitor. It is under phase II clinical investigation (NCT02454842, clinicaltrials.gov) for treating HER1 mutant T790M-negative NSCLC subjects who have progressed on HER1-TKI [151].

5.17. TAK-285

TAK-285 is a dual, irreversible and specific drug candidate exhibiting kinase inhibitory activity against HER1/HER2 with IC_{50} values of 23/17 nM, respectively. It is a non p-glycoprotein (Pgp) substrate unlike lapatinib and can penetrate central nervous system efficiently, as demonstrated in rat and mouse tumor xenograft models. It has over-

come lapatinib's limitation of poor blood-brain-barrier penetration [152]. Unbound fraction of TAK-285 was detected in extracellular space of brain. This confirmed its substantial penetration in brain and potential for the treatment of brain metastases among HER2 positive subjects [153]. TAK-285 has completed phase I evaluation (NCT00535522, clinicaltrials.gov) in advanced cancer patients and the drug was found to be safe at maximum-tolerated dose. Its cerebrospinal fluid distribution was also confirmed, but the free concentration of the drug was lower than the required amount for the inhibition of biological target [154].

5.18. AEE788

AEE788 is a reversible dual inhibitor of HER1/HER2 tyrosine kinase displaying IC_{50} values of 2/6 nM. In addition, it also inhibits VEGFR-TK. It displayed good pharmacokinetic profile combined with potent antitumor activity against HER1 and HER2 overexpressing cell lines and mouse tumor xenograft models [155]. AEE788 alone, and in combination with everolimus, has completed phase I/II trials (NCT00116376 and NCT00107237, respectively, clinicaltrials.gov) for the treatment of patients with recurrent glioblastoma multiforme.

5.19. BMS-599626 (AC480)

BMS-599626 is a selective, dual HER1/HER2 inhibitor, showing excellent *in vivo* activity in HER1 and HER2 driven tumor models [156]. It was found to disrupt HER1 and HER2 signaling and retarded cell proliferation in tumor cell lines dependent on HER1/HER2 [157]. It inhibited HER1, HER2 and HER4 TKs with IC_{50} values of 22 nM, 32 nM and 190 nM, respectively. The compound is well-tolerated for advanced solid tumors [158]. It has completed phase I study (NCT00207012, clinicaltrials.gov) in patients with HER2-expressing advanced solid malignancies.

5.20. S-222611

S-222611 is an oral, reversible, potent HER1/HER2 inhibitor showing IC_{50} values below 10 nM for both kinases [159]. It exhibited anti-proliferative effects against HER1 and/or HER2 overexpressing human breast cancer cell lines (BT474, SKBR3, MDA-MB-175VII, MDA-MB-453) and human gastric cancer cell line (NCI-N87) with IC_{50} values ranging from 8.3 to 48.6 nM. It was found to be 3 to 5-fold more potent than lapatinib. Tumor-inhibitory effects were also seen in xenograft models [160]. Shrinkage of brain metastases as a consequence of S-222611 treatment, was seen among HER2 positive metastatic breast cancer patients. Phase-I dose-escalation study acclaimed a daily dose of 800 mg of S-222611 [161].

5.21. Pirotinib (KBP-5209)

Pirotinib is an orally bioavailable, irreversible and selective inhibitor of the EGFR family receptors *i.e.* HER1, HER2 and HER4, with potential antineoplastic activity [162]. Recruitment of participants is ongoing for phase I study of pirotinib (NCT02442414, www.clinicaltrials.gov) in patients with advanced solid tumors to evaluate safety and tolerability of the drug [163].

CONCLUSION

HER2 overexpression is associated with the aggression and increased chances of metastasis of breast cancer cases. Discovery of monoclonal antibody, trastuzumab helped in HER2 targeted treatment of breast cancer. Antibody-based therapy is the first line treatment of HER2 overexpressing breast cancer. TKIs were developed as alternative therapeutic agents for HER2 positive breast cancer. These proved effective for treating central nervous system metastases in HER2 positive breast cancer due to their ability to cross the BBB. Development of drug resistance and relapse remained limiting factors with these treatment strategies. There are scopes for the improvement of TKIs based treatment strategies. A number of novel TKIs are under various phases of clinical development for the treatment of HER2 positive breast cancer. An effective drug candidate is desired that can selectively target HER2 and show minimum toxicity, overcome drug resistance and other side effects.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] McGuire, A.; Brown, J. A.; Malone, C.; McLaughlin, R.; Kerin, M. J. effects of age on the detection and management of breast cancer. *Cancers,* **2015**, *7*(2), 908-929.

[2] Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2015. *CA Cancer J. Clin.,* **2015**, *65*(1), 5-29.

[3] Giuliano, M.; Trivedi, M.V.; Schiff, R. Bidirectional crosstalk between the estrogen receptor and human epidermal growth factor receptor 2 signaling pathways in breast cancer: molecular basis and clinical implications. *Breast Care,* **2013**, *8*(4), 256-262.

[4] Iqbal, N.; Iqbal, N. Human epidermal growth factor receptor 2 (HER2) in cancers: overexpression and therapeutic implications. *Mol. Biol. Int.*, **2014**, *2014*.

[5] Gutierrez, C.; Schiff, R. HER 2: biology, detection, and clinical implications. *Arch. Pathol. Lab. Med.*, **2011**, *135*(1), 55-62.

[6] Brennan, P.J.; Kumagai, T.; Berezov, A.; Murali, R.; Greene, M.I. HER2/neu: mechanisms of dimerization/oligomerization. *Oncogene,* **2000**, *19*(53), 6093-6101.

[7] Park, J.W.; Neve, R.M.; Szollosi, J.; Benz, C.C. Unraveling the biologic and clinical complexities of HER2. *Clin. Breast Cancer,* **2008**, *8*(5), 392-401.

[8] Zhou, Z.; Hick, D.G. HER2 Amplification or overexpression in upper gi tract and breast cancer with clinical diagnosis and treatment In: *Oncogene and Cancer- From Bench to Clinic;* Yahwardiah Siregar (Ed.); InTech, **2014**, doi: 10.5772/53921.

[9] Eccles, S.A. The epidermal growth factor receptor/Erb-B/HER family in normal and malignant breast biology. *Int. J. Dev. Biol.*, **2011**, *55*(7), 685-696.

[10] Lee, C.-C.; Yang, H.-L.; Way, T.-D.; Kumar, K.; Juan, Y.-C.; Cho, H.-J.; Lin, K.-Y.; Hsu, L.-S.; Chen, S.-C.; Hseu, Y.-C. Inhibition of cell growth and induction of apoptosis by Antrodia camphorata in HER-2/neu-overexpressing breast cancer cells through the induction of ROS, depletion of HER-2/neu, and disruption of the

PI3K/Akt signaling pathway. *Evid. Based Complement. Alternat. Med.*, **2012**, *2012*.

[11] Tai, W.; Mahato, R.; Cheng, K. The role of HER2 in cancer therapy and targeted drug delivery. *J. Control. Release,* **2010**, *146*(3), 264-275.

[12] Ross, J.S.; Slodkowska, E.A.; Symmans, W.F.; Pusztai, L.; Ravdin, P.M.; Hortobagyi, G.N. The HER-2 receptor and breast cancer: ten years of targeted anti–HER-2 therapy and personalized medicine. *Oncologist*, **2009**, *14*(4), 320-368.

[13] Roskoski, R. The ErbB/HER receptor protein-tyrosine kinases and cancer. *Biochem. Biophys. Res. Commun.*, **2004**, *319*(1), 1-11.

[14] Gutierrez, C.; Schiff, R. HER2: biology, detection, and clinical implications. *Arch. Pathol. Lab. Med.,* **2011**, *135*(1), 55-62.

[15] Moasser, M.M.; Krop, I.E. The evolving landscape of HER2 targeting in breast cancer. *JAMA Oncol.,* **2015**, *1*(8), 1154-1161.

[16] Hurvitz, S.A.; Hu, Y.; O'Brien, N.; Finn, R.S. Current approaches and future directions in the treatment of HER2-positive breast cancer. *Cancer Treat Rev.*, **2013**, *39*(3), 219-229.

[17] Lin, N.U.; Carey, L.A.; Liu, M.C.; Younger, J.; Come, S.E.; Ewend, M.; Harris, G.J.; Bullitt, E.; Van den Abbeele, A.D.; Henson, J.W. Phase II trial of lapatinib for brain metastases in patients with human epidermal growth factor receptor 2–positive breast cancer. *J. Clin. Oncol.*, **2008**, *26*(12), 1993-1999.

[18] Xia, W.; Liu, L.-H.; Ho, P.; Spector, N.L. Truncated ErbB2 receptor (p95ErbB2) is regulated by heregulin through heterodimer formation with ErbB3 yet remains sensitive to the dual EGFR/ErbB2 kinase inhibitor GW572016. *Oncogene*, **2004**, *23*(3), 646-653.

[19] Aertgeerts, K.; Skene, R.; Yano, J.; Sang, B.-C.; Zou, H.; Snell, G.; Jennings, A.; Iwamoto, K.; Habuka, N.; Hirokawa, A. Structural analysis of the mechanism of inhibition and allosteric activation of the kinase domain of HER2 protein. *J. Biol. Chem.*, **2011**, *286*(21), 18756-18765.

[20] Cohen, B.D.; Kiener, P.A.; Green, J.M.; Foy, L.; Fell, H.P.; Zhang, K. The relationship between human epidermal growth-like factor receptor expression and cellular transformation in NIH3T3 cells. J. *Biol. Chem.*, **1996**, *271*(48), 30897-30903.

[21] Brignola, P.S.; Lackey, K.; Kadwell, S.H.; Hoffman, C.; Horne, E.; Carter, H.L.; Stuart, J.D.; Blackburn, K.; Moyer, M.B.; Alligood, K.J. Comparison of the biochemical and kinetic properties of the type 1 receptor tyrosine kinase intracellular domains- demonstration of differential sensitivity to kinase inhibitors. *J. Biol. Chem.,* **2002**, *277*(2), 1576-1585.

[22] Fan, Y.-X.; Wong, L.; Ding, J.; Spiridonov, N.A.; Johnson, R.C.; Johnson, G.R. Mutational activation of ErbB2 reveals a new protein kinase autoinhibition mechanism. *J. Biol. Chem.,* **2008**, *283*(3), 1588-1596.

[23] Stephens, P.; Hunter, C.; Bignell, G.; Edkins, S.; Davies, H.; Teague, J.; Stevens, C.; O'meara, S.; Smith, R.; Parker, A. Lung cancer: intragenic ERBB2 kinase mutations in tumours. *Nature*, **2004**, *431*(7008), 525-526.

[24] Lee, J.W.; Soung, Y.H.; Seo, S.H.; Kim, S.Y.; Park, C.H.; Wang, Y.P.; Park, K.; Nam, S.W.; Park, W.S.; Kim, S.H. Somatic mutations of ERBB2 kinase domain in gastric, colorectal, and breast carcinomas. *Clin. Cancer Res.*, **2006**, *12*(1), 57-61.

[25] Wang, S.E.; Narasanna, A.; Perez-Torres, M.; Xiang, B.; Wu, F. Y.; Yang, S.; Carpenter, G.; Gazdar, A.F.; Muthuswamy, S.K.; Arteaga, C.L. HER2 kinase domain mutation results in constitutive phosphorylation and activation of HER2 and EGFR and resistance to EGFR tyrosine kinase inhibitors. *Cancer Cell*, **2006**, *10*(1), 25-38.

[26] Shigematsu, H.; Takahashi, T.; Nomura, M.; Majmudar, K.; Suzuki, M.; Lee, H.; Wistuba, I.I.; Fong, K.M.; Toyooka, S.; Shimizu, N. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. *Cancer Res.*, **2005**, *65*(5), 1642-1646.

[27] Emsley, P.; Lohkamp, B.; Scott, W.G.; Cowtan, K. Features and development of Coot. *Acta Crystallogr. Sect D- Biol. Crystallogr.*, **2010**, *66*(4), 486-501.

[28] Zhang, X.; Gureasko, J.; Shen, K.; Cole, P.A.; Kuriyan, J. An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. *Cell*, **2006**, *125*(6), 1137-1149.

[29] Zhang, X.; Pickin, K.A.; Bose, R.; Jura, N.; Cole, P.A.; Kuriyan, J. Inhibition of the EGF receptor by binding of MIG6 to an activating kinase domain interface. *Nature*, **2007**, *450*(7170), 741-744.

[30] Qiu, C.; Tarrant, M.K.; Choi, S.H.; Sathyamurthy, A.; Bose, R.; Banjade, S.; Pal, A.; Bornmann, W.G.; Lemmon, M.A.; Cole, P. A., Mechanism of activation and inhibition of the HER4/ErbB4 kinase. *Structure*, **2008**, *16*(3), 460-467.

[31] Vu, T.; Claret, F.X. Trastuzumab: updated mechanisms of action and resistance in breast cancer. *Front. Oncol.,* **2012**, *2*(article 62), 1-6.

[32] Arteaga, C.L.; Sliwkowski, M.X.; Osborne, C.K.; Perez, E.A.; Puglisi, F.; Gianni, L. Treatment of HER2-positive breast cancer: current status and future perspectives. *Nat. Rev. Clin. Oncol.*, **2012**, *9*(1), 16-32.

[33]. Capelan, M.; Pugliano, L.; De Azambuja, E.; Bozovic, I.; Saini, K.; Sotiriou, C.; Loi, S.; Piccart-Gebhart, M. Pertuzumab: new hope for patients with HER2-positive breast cancer. *Ann. Oncol.*, **2013**, *24*(2), 273-282.

[34] Amiri-Kordestani, L.; Blumenthal, G.M.; Xu, Q.C.; Zhang, L.; Tang, S.W.; Ha, L.; Weinberg, W.C.; Chi, B.; Candau-Chacon, R.; Hughes, P. FDA approval: ado-trastuzumab emtansine for the treatment of patients with HER2-positive metastatic breast cancer. *Clin. Cancer Res.*, **2014**, *20*(17), 4436-4441.

[35] Verma, S.; Miles, D.; Gianni, L.; Krop, I.E.; Welslau, M.; Baselga, J.; Pegram, M.; Oh, D.-Y.; Diéras, V.; Guardino, E. Trastuzumab emtansine for HER2-positive advanced breast cancer. *N. Engl. J. Med.*, **2012**, *367*(19), 1783-1791.

[36] Nielsen, D.L.; Kümler, I.; Palshof, J.A.; Andersson, M. Efficacy of HER2-targeted therapy in metastatic breast cancer. Monoclonal antibodies and tyrosine kinase inhibitors. *Breast*, **2013**, *22*(1), 1-12.

[37] Bailey, T.A.; Luan, H.; Clubb, R.J.; Naramura, M.; Band, V.; Raja, S.M.; Band, H. Mechanisms of Trastuzumab resistance in ErbB2-driven breast cancer and newer opportunities to overcome therapy resistance. *J. Carcinogenesis,* **2011**, *10*(1), 28.

[38] Spector, N.; Xia, W.; El-Hariry, I.; Yarden, Y.; Bacus, S. HER2 therapy. Small molecule HER-2 tyrosine kinase inhibitors. *Breast Cancer Res.*, **2007**, *9*(2), *205*, 1-8.

[39] Schroeder, R.L.; Stevens, C.L.; Sridhar, J. Small molecule tyrosine kinase inhibitors of ErbB2/HER2/Neu in the treatment of aggressive breast cancer. *Molecules*, **2014**, *19*(9), 15196-15212.

[40] Zardavas, D.; Cameron, D.; Krop, I.; Piccart-Gebhart, M. Beyond trastuzumab and lapatinib: New options for HER2-positive breast cancer. *J. Clin. Oncol.,* **2013**.

[41] Tsang, R.; Finn, R. Beyond trastuzumab: novel therapeutic strategies in HER2-positive metastatic breast cancer. *Br. J. Cancer,* **2012**, *106*(1), 6-13.

[42] Vrbic, S.; Pejcic, I.; Filipovic, S.; Kocic, B.; Vrbic, M. Current and future anti-HER2 therapy in breast cancer. *J. BUON.*, **2013**, *18*(1), 4-16.

[43] Saini, K.S.; Azim, H.A.; Metzger-Filho, O.; Loi, S.; Sotiriou, C.; de Azambuja, E.; Piccart, M. Beyond trastuzumab: new treatment options for HER2-positive breast cancer. *Breast*, **2011**, *20*, S20-S27.

[44] Gradishar, W. Emerging approaches for treating HER2-positive metastatic breast cancer beyond trastuzumab. *Ann. Oncol.,* **2013**, *24*(10), 2492-2500.

[45] Lippa, B.; Kauffman, G.S.; Arcari, J.; Kwan, T.; Chen, J.; Hungerford, W.; Bhattacharya, S.; Zhao, X.; Williams, C.; Xiao, J. The discovery of highly selective erbB2 (HER2) inhibitors for the treatment of cancer. *Bioorg. Med. Chem. Lett.*, **2007**, *17*(11), 3081-3086.

[46] Rachid, Z.; MacPhee, M.; Williams, C.; Todorova, M.; Jean-Claude, B.J. Design and synthesis of new stabilized combitriazenes for targeting solid tumors expressing the epidermal growth factor receptor (EGFR) or its closest homologue HER2. *Bioorg. Med. Chem. Lett.*, **2009**, *19*(18), 5505-5509.

[47] Mahboobi, S.; Sellmer, A.; Winkler, M.; Eichhorn, E.; Pongratz, H.; Ciossek, T.; Baer, T.; Maier, T.; Beckers, T. Novel chimeric histone deacetylase inhibitors: a series of lapatinib hybrides as potent inhibitors of epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), and histone deacetylase activity. *J. Med. Chem.*, **2010**, *53*(24), 8546-8555.

[48] Wu, X.; Li, M.; Qu, Y.; Tang, W.; Zheng, Y.; Lian, J.; Ji, M.; Xu, L. Design and synthesis of novel Gefitinib analogs with improved anti-tumor activity. *Bioorg. Med. Chem.*, **2010**, *18*(11), 3812-3822.

[49] Beckers, T.; Mahboobi, S.; Sellmer, A.; Winkler, M.; Eichhorn, E.; Pongratz, H.; Maier, T.; Ciossek, T.; Baer, T.; Kelter, G. Chimerically designed HDAC-and tyrosine kinase inhibitors. A series of erlotinib hybrids as dual-selective inhibitors of EGFR, HER2 and histone deacetylases. *Med. Chem. Comm.*, **2012**, *3*(7), 829-835.

[50] Li, D.-D.; Qin, Y.-J.; Sun, J.; Li, J.-R.; Fang, F.; Du, Q.-R.; Qian, Y.; Gong, H.-B.; Zhu, H.-L. Optimization of substituted 6-salicyl-

4-anilinoquinazoline derivatives as dual EGFR/HER2 tyrosine kinase inhibitors. *PloS One*, **2013**, *8*(8), 1-11.

[51] Zhang, L.; Fan, C.; Guo, Z.; Li, Y.; Zhao, S.; Yang, S.; Yang, Y.; Zhu, J.; Lin, D. Discovery of a potent dual EGFR/HER-2 inhibitor L-2 (selatinib) for the treatment of cancer. *Eur. J. Med. Chem.*, **2013**, *69*, 833-841.

[52] Ahmed, M.; Sadek, M.M.; Serrya, R.A.; Kafafy, A.-H. N.; Abouzid, K.A.; Wang, F. Assessment of new anti-HER2 ligands using combined docking, QM/MM scoring and MD simulation. *J. Mol. Graph. Model*, **2013**, *40*, 91-98.

[53] Ahmed, M.; Sadek, M.M.; Abouzid, K.A.; Wang, F. In silico design: extended molecular dynamic simulations of a new series of dually acting inhibitors against EGFR and HER2. *J. Mol. Graph. Model,* **2013**, *44*, 220-231.

[54] Sadek, M.M.; Serrya, R.A.; Kafafy, A.-H.N.; Ahmed, M.; Wang, F.; Abouzid, K.A. Discovery of new HER2/EGFR dual kinase inhibitors based on the anilinoquinazoline scaffold as potential anticancer agents. *J. Enzyme Inhib. Med. Chem.,* **2014**, *29*(2), 215-222.

[55] Elkamhawy, A.; Farag, A.K.; Viswanath, A.N.; Bedair, T.M.; Leem, D.G.; Lee, K.T.; Pae, A.N.; Roh, E.J. Targeting EGFR/HER2 tyrosine kinases with a new potent series of 6-substituted 4-anilinoquinazoline hybrids: Design, synthesis, kinase assay, cell-based assay, and molecular docking. *Bioorg. Med. Chem. Lett.*, **2015**, *25*(22), 5147-5154.

[56] Lyu, A.; Fang, L.; Gou, S. Design and synthesis of Lapatinib derivatives containing a branched side chain as HER1/HER2 targeting antitumor drug candidates. *Eur. J. Med. Chem.,* **2014**, *87*, 631-642.

[57] Yin, S.; Tang, C.; Wang, B.; Zhang, Y.; Zhou, L.; Xue, L.; Zhang, C. Design, synthesis and biological evaluation of novel EGFR/HER2 dual inhibitors bearing a oxazolo [4, 5-g] quinazolin-2 (1H)-one scaffold. *Eur. J. Med. Chem.*, **2016**, *120*, 26-36.

[58] Mastalerz, H.; Chang, M.; Chen, P.; Dextraze, P.; Fink, B.E.; Gavai, A.; Goyal, B.; Han, W.-C.; Johnson, W.; Langley, D. New C-5 substituted pyrrolotriazine dual inhibitors of EGFR and HER2 protein tyrosine kinases. *Bioorg. Med. Chem. Lett.*, **2007**, *17*(7), 2036-2042.

[59] Mastalerz, H.; Chang, M.; Gavai, A.; Johnson, W.; Langley, D.; Lee, F.Y.; Marathe, P.; Mathur, A.; Oppenheimer, S.; Tarrant, J. Novel C-5 aminomethyl pyrrolotriazine dual inhibitors of EGFR and HER2 protein tyrosine kinases. *Bioorg. Med. Chem. Lett.*, **2007**, *17*(10), 2828-2833.

[60] Mastalerz, H.; Chang, M.; Chen, P.; Fink, B.E.; Gavai, A.; Han, W.-C.; Johnson, W.; Langley, D.; Lee, F.Y.; Leavitt, K. 5-((4-Aminopiperidin-1-yl) methyl) pyrrolotriazine dual inhibitors of EGFR and HER2 protein tyrosine kinases. *Bioorg. Med. Chem. Lett.*, **2007**, *17*(17), 4947-4954.

[61] Ducray, R.; Ballard, P.; Barlaam, B.C.; Hickinson, M.D.; Kettle, J.G.; Ogilvie, D.J.; Trigwell, C.B. Novel 3-alkoxy-1H-pyrazolo [3, 4-d] pyrimidines as EGFR and erbB2 receptor tyrosine kinase inhibitors. *Bioorg. Med. Chem. Lett.*, **2008**, *18*(3), 959-962.

[62] Kawakita, Y.; Miwa, K.; Seto, M.; Banno, H.; Ohta, Y.; Tamura, T.; Yusa, T.; Miki, H.; Kamiguchi, H.; Ikeda, Y. Design and synthesis of pyrrolo [3, 2-d] pyrimidine HER2/EGFR dual inhibitors: Improvement of the physicochemical and pharmacokinetic profiles for potent *in vivo* anti-tumor efficacy. *Bioorg. Med. Chem.*, **2012**, *20*(20), 6171-6180.

[63] Kawakita, Y.; Banno, H.; Ohashi, T.; Tamura, T.; Yusa, T.; Nakayama, A.; Miki, H.; Iwata, H.; Kamiguchi, H.; Tanaka, T. Design and synthesis of pyrrolo [3, 2-d] pyrimidine human epidermal growth factor receptor 2 (HER2)/epidermal growth factor receptor (EGFR) dual inhibitors: exploration of novel back-pocket binders. *J. Med. Chem.*, **2012**, *55*(8), 3975-3991.

[64] Hubbard, R.D.; Dickerson, S.H.; Emerson, H.K.; Griffin, R.J.; Reno, M.J.; Hornberger, K.R.; Rusnak, D.W.; Wood, E.R.; Uehling, D.E.; Waterson, A.G. Dual EGFR/ErbB-2 inhibitors from novel pyrrolidinyl-acetylenic thieno [3, 2-d] pyrimidines. *Bioorg. Med. Chem. Lett.*, **2008**, *18*(21), 5738-5740.

[65] Waterson, A.G.; Petrov, K.G.; Hornberger, K.R.; Hubbard, R.D.; Sammond, D.M.; Smith, S.C.; Dickson, H.D.; Caferro, T.R.; Hinkle, K.W.; Stevens, K.L. Synthesis and evaluation of aniline headgroups for alkynyl thienopyrimidine dual EGFR/ErbB-2 kinase inhibitors. *Bioorg. Med. Chem. Lett.*, **2009**, *19*(5), 1332-1336.

[66] Rheault, T.R.; Caferro, T.R.; Dickerson, S.H.; Donaldson, K.H.; Gaul, M.D.; Goetz, A.S.; Mullin, R.J.; McDonald, O.B.; Petrov, K. G.; Rusnak, D.W. Thienopyrimidine-based dual EGFR/ErbB-2 inhibitors. *Bioorg. Med. Chem. Lett.*, **2009**, *19*(3), 817-820.

[67] Xu, G.; Abad, M.C.; Connolly, P.J.; Neeper, M.P.; Struble, G.T.; Springer, B.A.; Emanuel, S.L.; Pandey, N.; Gruninger, R.H.; Adams, M. 4-Amino-6-arylamino-pyrimidine-5-carbaldehyde hydrazones as potent ErbB-2/EGFR dual kinase inhibitors. *Bioorg. Med. Chem. Lett.*, **2008**, *18*(16), 4615-4619.

[68] Hughes, T.V.; Xu, G.; Wetter, S.K.; Connolly, P.J.; Emanuel, S.L.; Karnachi, P.; Pollack, S.R.; Pandey, N.; Adams, M.; Moreno-Mazza, S. A novel 5-[1, 3, 4-oxadiazol-2-yl]-N-aryl-4, 6-pyrimidine diamine having dual EGFR/HER2 kinase activity: Design, synthesis, and biological activity. *Bioorg. Med. Chem. Lett.,* **2008**, *18*(17), 4896-4899.

[69] Xu, G.; Searle, L.L.; Hughes, T.V.; Beck, A.K.; Connolly, P.J.; Abad, M.C.; Neeper, M.P.; Struble, G.T.; Springer, B.A.; Emanuel, S.L. Discovery of novel 4-amino-6-arylaminopyrimidine-5-carbaldehyde oximes as dual inhibitors of EGFR and ErbB-2 protein tyrosine kinases. *Bioorg. Med. Chem. Lett.*, **2008**, *18*(12), 3495-3499.

[70] Suzuki, N.; Shiota, T.; Watanabe, F.; Haga, N.; Murashi, T.; Ohara, T.; Matsuo, K.; Omori, N.; Yari, H.; Dohi, K. Discovery of novel 5-alkynyl-4-anilinopyrimidines as potent, orally active dual inhibitors of EGFR and Her-2 tyrosine kinases. *Bioorg. Med. Chem. Lett.*, **2012**, *22*(1), 456-460.

[71] Kawakita, Y.; Seto, M.; Ohashi, T.; Tamura, T.; Yusa, T.; Miki, H.; Iwata, H.; Kamiguchi, H.; Tanaka, T.; Sogabe, S. Design and synthesis of novel pyrimido [4, 5-b] azepine derivatives as HER2/EGFR dual inhibitors. *Bioorg. Med. Chem. Lett.*, **2013**, *21*(8), 2250-2261.

[72] Cheng, Z.-Y.; Li, W.-J.; He, F.; Zhou, J.-M.; Zhu, X.-F. Synthesis and biological evaluation of 4-aryl-5-cyano-2H-1, 2, 3-triazoles as inhibitor of HER2 tyrosine kinase. *Bioorg. Med. Chem.*, **2007**, *15*(3), 1533-1538.

[73] Gundla, R.; Kazemi, R.; Sanam, R.; Muttineni, R.; Sarma, J.A.; Dayam, R.; Neamati, N. Discovery of novel small-molecule inhibitors of human epidermal growth factor receptor-2: Combined ligand and target-based approach. *J. Med. Chem.*, **2008**, *51*(12), 3367-3377.

[74] Ding, N.; Zhang, W.; Xiao, H. L.; Wang, P.; Li, Y. X. Synthesis and biological evaluation of a series of novel salicylanilides as inhibitors of EGFR protein tyrosine kinases. *Chin. Chem. Lett.*, **2012**, *23*(5), 529-532.

[75] Wang, H.-H.; Qiu, K.-M.; Cui, H.-E.; Yang, Y.-S.; Xing, M.; Qiu, X.-Y.; Bai, L.-F.; Zhu, H.-L. Synthesis, molecular docking and evaluation of thiazolyl-pyrazoline derivatives containing benzodioxole as potential anticancer agents. *Bioorg. Med. Chem.*, **2013**, *21*(2), 448-455.

[76] Sridhar, J.; Sfondouris, M.E.; Bratton, M.R.; Nguyen, T.-L. K.; Townley, I.; Stevens, C.L.K.; Jones, F.E. Identification of quinones as HER2 inhibitors for the treatment of trastuzumab resistant breast cancer. *Bioorg. Med. Chem. Lett.*, **2014**, *24*(1), 126-131.

[77] Sangani, C.B.; Makawana, J.A.; Duan, Y.-T.; Yin, Y.; Teraiya, S.B.; Thumar, N.J.; Zhu, H.-L. Design, synthesis and molecular modeling of biquinoline–pyridine hybrids as a new class of potential EGFR and HER-2 kinase inhibitors. *Bioorg. Med. Chem. Lett.*, **2014**, *24*(18), 4472-4476.

[78] Elseginy, S.A.; Lazaro, G.; Nawwar, G.A.; Amin, K.M.; Hiscox, S.; Brancale, A. Computer-aided identification of novel anticancer compounds with a possible dual HER1/HER2 inhibition mechanism. *Bioorg. Med. Chem. Lett.*, **2015**, *25*(4), 758-762.

[79] Zalloum, H.; Tayyem, R.; Bustanji, Y.; Zihlif, M.; Mohammad, M.; Rjai, T.A.; Mubarak, M.S. Discovery of new human epidermal growth factor receptor-2 (HER2) inhibitors for potential use as anticancer agents *via* ligand-based pharmacophore modeling. *J. Mol. Graph. Model.*, **2015**, *61*, 61-84.

[80] Tao, X.-X.; Duan, Y.-T.; Chen, L.-W.; Tang, D.-J.; Yang, M.-R.; Wang, P.-F.; Xu, C.; Zhu, H.-L. Design, synthesis and biological evaluation of pyrazolyl-nitroimidazole derivatives as potential EGFR/HER-2 kinase inhibitors. *Bioorg. Med. Chem. Lett.*, **2016**, *26*(2), 677-683.

[81] Segovia-Mendoza, M.; González-González, M.E.; Barrera, D.; Díaz, L.; García-Becerra, R. Efficacy and mechanism of action of the tyrosine kinase inhibitors gefitinib, lapatinib and neratinib in the treatment of HER2-positive breast cancer: preclinical and clinical evidence. *Am. J. Cancer Res.*, **2015**, *5*(9), 2531.

[82] Xia, W.; Gerard, C.M.; Liu, L.; Baudson, N.M.; Ory, T.L.; Spector, N.L. Combining lapatinib (GW572016), a small molecule inhibitor of ErbB1 and ErbB2 tyrosine kinases, with therapeutic anti-ErbB2 antibodies enhances apoptosis of ErbB2-overexpressing breast cancer cells. *Oncogene*, **2005**, *24*(41), 6213-6221.

[83] Xia, W.; Mullin, R.J.; Keith, B.R.; Liu, L.-H.; Ma, H.; Rusnak, D.W.; Owens, G.; Alligood, K.J.; Spector, N.L. Anti-tumor activity of GW572016: a dual tyrosine kinase inhibitor blocks EGF activation of EGFR/erbB2 and downstream Erk1/2 and AKT pathways. *Oncogene*, **2002**, *21*(41), 6255-6263.

[84] Moy, B.; Kirkpatrick, P.; Kar, S.; Goss, P. Lapatinib. *Nat. Rev. Drug Discov.*, **2007**, *6*(6), 431-432.

[85] Ryan, Q.; Ibrahim, A.; Cohen, M.H.; Johnson, J.; Ko, C.-W.; Sridhara, R.; Justice, R.; Pazdur, R. FDA drug approval summary: lapatinib in combination with capecitabine for previously treated metastatic breast cancer that overexpresses HER-2. *Oncologist*, **2008**, *13*(10), 1114-1119.

[86] Guarneri, V. Lapatinib plus letrozole for postmenopausal patients with advanced HER2+/HR+ breast cancer. *Expert Rev. Anticancer Ther.*, **2009**, *9*(11), 1549-1557.

[87] Schwartzberg, L.S.; Franco, S.X.; Florance, A.; O'Rourke, L.; Maltzman, J.; Johnston, S. Lapatinib plus letrozole as first-line therapy for HER-2+ hormone receptor–positive metastatic breast cancer. *Oncologist*, **2010**, *15*(2), 122-129.

[88] Burris, H.A.; Hurwitz, H.I.; Dees, E.C.; Dowlati, A.; Blackwell, K. L.; O'Neil, B.; Marcom, P.K.; Ellis, M.J.; Overmoyer, B.; Jones, S. F. Phase I safety, pharmacokinetics, and clinical activity study of lapatinib (GW572016), a reversible dual inhibitor of epidermal growth factor receptor tyrosine kinases, in heavily pretreated patients with metastatic carcinomas. *J. Clin. Oncol.*, **2005**, *23*(23), 5305-5313.

[89] Sergina, N.V.; Rausch, M.; Wang, D.; Blair, J.; Hann, B.; Shokat, K.M.; Moasser, M.M. Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. *Nature,* **2007**, *445*(7126), 437-441.

[90] Xia, W.; Bacus, S.; Hegde, P.; Husain, I.; Strum, J.; Liu, L.; Paulazzo, G.; Lyass, L.; Trusk, P.; Hill, J. A model of acquired autoresistance to a potent ErbB2 tyrosine kinase inhibitor and a therapeutic strategy to prevent its onset in breast cancer. *Proc. Natl. Acad. Sci.*, **2006**, *103*(20), 7795-7800.

[91] Peacock, N.; Jones, S.; Yardley, D.; Bendell, J.; Infante, J.; Murphy, P.; Burris III, H. A phase I study of panobinostat (LBH589) with capecitabine with or without lapatinib. *J. Clin. Oncol.*, **2010**, *28*(15), 1115-1115.

[92] Aljada, A.; Mousa, S.A. Metformin and neoplasia: implications and indications. *Pharmacol. Ther.,* **2012**, *133*(1), 108-115.

[93] Yardley, D.A.; Hainsworth, J.D.; Hamilton, E.P.; Hart, L.L.; Shastry, M.; DeBusk, L.M.; Burris, H.A. A phase II study with lead-in safety cohort of cabazitaxel (C) plus lapatinib (L) as therapy for HER2+ metastatic breast cancer (MBC) with intracranial metastases (mets). *Cancer Res.,* **2014**, *73*, OT1-1-13.

[94] Collins, D.; Hill, A.; Young, L. Lapatinib: A competitor or companion to trastuzumab? *Cancer Treat. Rev.,* **2009**, *35*(7), 574-581.

[95] Iyengar, N.M.; Fornier, M.N.; Sugarman, S.; Theodoulou, M.; Troso-Sandoval, T.A.; D'Andrea, G.; Drullinsky, P.; Fasano, J.; Gajria, D.; Goldfarb, S.B. Phase II feasibility study of paclitaxel (T) with trastuzumab (H) and lapatinib (L) for node-negative, HER2-positive breast cancer (BC). *J. Clin. Oncol.,* **2014***, 32*(15)*, 633-633.*

[96] Baselga, J.; Bradbury, I.; Eidtmann, H.; Di Cosimo, S.; de Azambuja, E.; Aura, C.; Gómez, H.; Dinh, P.; Fauria, K.; Van Dooren, V. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. *Lancet*, **2012**, *379*(9816), 633-640.

[97] Hirsh, V. Afatinib (BIBW 2992) development in non-small-cell lung cancer. *Future Oncol*., **2011**, *7*(7), 817-825.

[98] Geuna, E.; Montemurro, F.; Aglietta, M.; Valabrega, G. Potential of afatinib in the treatment of patients with HER2-positive breast cancer. *Breast Cancer*, **2012**, *4*, 131-137.

[99] Solca, F.; Dahl, G.; Zoephel, A.; Bader, G.; Sanderson, M.; Klein, C.; Kraemer, O.; Himmelsbach, F.; Haaksma, E.; Adolf, G. R. Target binding properties and cellular activity of afatinib (BIBW 2992), an irreversible ErbB family blocker. *J. Pharm. Exp. Ther.*, **2012**, *343*(2), 342-350.

[100] Kim, S.M.; Kwon, O.-J.; Hong, Y.K.; Kim, J.H.; Solca, F.; Ha, S.J.; Soo, R.A.; Christensen, J.G.; Lee, J.H.; Cho, B.C. Activation of IL-6R/JAK1/STAT3 signaling induces *de novo* resistance to irreversible egfr inhibitors in non–small cell lung cancer with T790M Resistance Mutation. *Mol. Cancer Ther.*, **2012**, *11*(10), 2254-2264.

[101] Lee, H.-J.; Schaefer, G.; Heffron, T.P.; Shao, L.; Ye, X.; Sideris, S.; Malek, S.; Chan, E.; Merchant, M.; La, H. Noncovalent wild-type–sparing inhibitors of EGFR T790M. *Cancer Discov.*, **2013**, *3*(2), 168-181.

[102] Mack, P.C.; Goodwin, N.; Holland, W.S.; Kelly, K.; Lara, P.; Gandara, D.R. Differential activity of afatinib (AFAT), cetuximab (CET), and erlotinib (E) in a patient-derived xenograft (PDX) model of acquired E resistance. *J. Clin. Oncol.*, **2013**, *31*(15), 8110-8110.

[103] Ninomiya, T.; Takigawa, N.; Ichihara, E.; Ochi, N.; Murakami, T.; Honda, Y.; Kubo, T.; Minami, D.; Kudo, K.; Tanimoto, M. Afatinib prolongs survival compared with gefitinib in an epidermal growth factor receptor-driven lung cancer model. *Mol. Cancer Ther.*, **2013**, *12*(5), 589-597.

[104] Regales, L.; Gong, Y.; Shen, R.; de Stanchina, E.; Vivanco, I.; Goel, A.; Koutcher, J.A.; Spassova, M.; Ouerfelli, O.; Mellinghoff, I.K. Dual targeting of EGFR can overcome a major drug resistance mutation in mouse models of EGFR mutant lung cancer. *J. Clin. Invest.*, **2009**, *119*(10), 3000-3010.

[105] Tabara, K.; Kanda, R.; Sonoda, K.; Kubo, T.; Murakami, Y.; Kawahara, A.; Azuma, K.; Abe, H.; Kage, M.; Yoshinaga, A. Loss of activating EGFR mutant gene contributes to acquired resistance to EGFR tyrosine kinase inhibitors in lung cancer cells. *PLoS One*, **2012**, *7*(7), 1-16.

[106] Takezawa, K.; Okamoto, I.; Tanizaki, J.; Kuwata, K.; Yamaguchi, H.; Fukuoka, M.; Nishio, K.; Nakagawa, K. Enhanced anticancer effect of the combination of BIBW2992 and thymidylate synthase–targeted agents in non–small cell lung cancer with the T790M mutation of epidermal growth factor receptor. *Mol. Cancer Ther.*, **2010**, *9*(6), 1647-1656.

[107] Joensuu, H.; Ould Kaci, M. LUX-breast 3: Randomized phase II study of afatinib alone or with vinorelbine versus investigator's choice of treatment in patients (pts) with HER2-positive breast cancer (BC) with progressive brain metastases (BM) after trastuzumab or lapatinib-based therapy. *J. Clin. Oncol.*, **2012**, *30*(15), 647-647.

[108] Swanton, C.; Cromer, J. Open-label, phase II trial of afatinib, with or without vinorelbine (V), for the treatment of HER2-overexpressing inflammatory breast cancer (IBC), *Cancer Res.,* **2012**, *72*(24), 1-14.

[109] Lin, N.U.; Winer, E.P.; Wheatley, D.; Carey, L.A.; Houston, S.; Mendelson, D.; Munster, P.; Frakes, L.; Kelly, S.; Garcia, A.A. A phase II study of afatinib (BIBW 2992), an irreversible ErbB family blocker, in patients with HER2-positive metastatic breast cancer progressing after trastuzumab. *Breast Cancer Res. Treat*, **2012**, *133*(3), 1057-1065.

[110] Rimawi, M.F.; Aleixo, S.B.; Rozas, A.A.; de Matos Neto, J.N.; Caleffi, M.; Figueira, A.C.; Souza, S.C.; Reiriz, A.B.; Gutierrez, C.; Arantes, H; Uttenreuther-Fischer, M.M. A neoadjuvant, randomized, open-label phase II trial of afatinib versus trastuzumab versus lapatinib in patients with locally advanced HER2-positive breast cancer. *Clin. Breast Cancer*, **2015**, *15*(2), 101-109.

[111] Hickish, T.; Mehta, A.; Jain, M.; Huang, C.; Kovalenko, N.; Udovitsa, D.; Pemberton, K.; Uttenreuther-Fischer, M.; Tseng, L.; Group, L.-B. S. Abstract OT1-1-17: LUX-Breast 2: Phase II, open-label study of oral afatinib in HER2-overexpressing metastatic breast cancer (MBC) patients (pts) who progressed on prior trastuzumab and/or lapatinib*. *Cancer Res.*, **2012**, *72*(24), 1-17. [112] Cortés, J.; Dieras, V.; Ro, J.; Barriere, J.; Bachelot, T; Hurvitz, S.; Le Rhun, E.; Espié, M.; Kim, S.B.; Schneeweiss, A.; Sohn, J.H. Afatinib alone or afatinib plus vinorelbine versus investigator's choice of treatment for HER2-positive breast cancer with progressive brain metastases after trastuzumab, lapatinib, or both (LUX-Breast 3): a randomised, open-label, multicentre, phase 2 trial. *Lancet Oncol.*, **2015**, *16*(16), 1700-1710.

[113] Hickinson, D.M.; Klinowska, T.; Speake, G.; Vincent, J.; Trigwell, C.; Anderton, J.; Beck, S.; Marshall, G.; Davenport, S.; Callis, R. AZD8931, an equipotent, reversible inhibitor of signaling by epidermal growth factor receptor, ERBB2 (HER2), and ERBB3: a unique agent for simultaneous ERBB receptor blockade in cancer. *Clin. Cancer Res.*, **2010**, *16*(4), 1159-1169.

[114] Barlaam, B.; Anderton, J.; Ballard, P.; Bradbury, R.H.; Hennequin, L.F.; Hickinson, D.M.; Kettle, J.G.; Kirk, G.; Klinowska, T.; Lambert-Van der Brempt, C. Discovery of azd8931, an equipotent, reversible inhibitor of signaling by egfr, HER2, and HER3 receptors. *ACS Med. Chem. Lett.*, **2013**, *4*(8), 742-746.

[115] Baselga, J.; Hegg, R.; Losada, M.V.; Vidaurre, T.; Lluch, A.; Petrakova, K.; Mann, H.; Ghiorghiu, S.; Stuart, M.; Landers, D. Abstract LB-146: A phase II randomized placebo-controlled study of AZD8931, an inhibitor of EGFR, HER2, and HER3 signaling, plus paclitaxel (P) vs P alone in patients (pts) with low HER2-expressing advanced breast cancer (BC)(THYME). *Cancer Res.*, **2013**, *73*(8), 146-146.

[116] Tsou, H.-R.; Overbeek-Klumpers, E.G.; Hallett, W.A.; Reich, M.F.; Floyd, M.B.; Johnson, B.D.; Michalak, R.S.; Nilakantan, R.; Discafani, C.; Golas, J. Optimization of 6, 7-disubstituted-4-(arylamino) quinoline-3-carbonitriles as orally active, irreversible inhibitors of human epidermal growth factor receptor-2 kinase activity. *J. Med. Chem*., **2005**, *48*(4), 1107-1131.

[117] Rabindran, S.K. Antitumor activity of HER-2 inhibitors. *Cancer Lett.*, **2005**, *227*(1), 9-23.

[118] Rabindran, S.K.; Discafani, C.M.; Rosfjord, E.C.; Baxter, M.; Floyd, M.B.; Golas, J.; Hallett, W.A.; Johnson, B.D.; Nilakantan, R.; Overbeek, E. Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase. *Cancer Res.*, **2004**, *64*(11), 3958-3965.

[119] Burstein, H.J.; Sun, Y.; Dirix, L.Y.; Jiang, Z.; Paridaens, R.; Tan, A.R.; Awada, A.; Ranade, A.; Jiao, S.; Schwartz, G. Neratinib, an irreversible ErbB receptor tyrosine kinase inhibitor, in patients with advanced ErbB2-positive breast cancer. *J. Clin. Oncol.*, **2010**, *28*(8), 1301-1307.

[120] Chan, A.; Delaloge, S.; Holmes, F.A.; Moy, B.; Iwata, H.; Harvey, V.J.; Robert, N.J.; Silovski, T.; Gokmen, E.; Von Minckwitz, G. Neratinib after adjuvant chemotherapy and trastuzumab in HER2-positive early breast cancer: Primary analysis at 2 years of a phase 3, randomized, placebo-controlled trial (ExteNET). *Lancet Oncol.*, **2016**, *17,* 366-367.

[121] Saura, C.; Garcia-Saenz, J.A.; Xu, B.; Harb, W.; Moroose, R.; Pluard, T.; Cortés, J.; Kiger, C.; Germa, C.; Wang, K. Safety and efficacy of neratinib in combination with capecitabine in patients with metastatic human epidermal growth factor receptor 2–positive breast cancer. *J. Clin. Oncol.*, **2014**, *32*(32), 3626-3633.

[122] Awada, A.; Dirix, L.; Sanchez, L.M.; Xu, B.; Luu, T.; Diéras, V.; Hershman, D.; Agrapart, V.; Ananthakrishnan, R.; Staroslawska, E. Safety and efficacy of neratinib (HKI-272) plus vinorelbine in the treatment of patients with ErbB2-positive metastatic breast cancer pretreated with anti-HER2 therapy. *Ann. Oncol.*, **2013**, *24*(1), 109-116.

[123] Martin, M.; Bonneterre, J.; Geyer, C.E.; Ito, Y.; Ro, J.; Lang, I.; Kim, S.-B.; Germa, C.; Vermette, J.; Wang, K. A phase two randomised trial of neratinib monotherapy versus lapatinib plus capecitabine combination therapy in patients with HER2+ advanced breast cancer. *Eur. J. Cancer*, **2013**, *49*(18), 3763-3772.

[124] Awada, A.; Colomer, R.; Inoue, K.; Bondarenko, I.; Badwe, R.A.; Demetriou, G.; Lee, S.C.; Mehta, A.O.; Kim, S.B.; Bachelot, T.; Goswami, C.; Neratinib plus paclitaxel vs trastuzumab plus paclitaxel in previously untreated metastatic ERBB2-positive breast cancer: the NEfERT-T randomized clinical trial. *JAMA Oncol.,* **2016**, *2*(12), 1557-1564.

[125] Ben-Baruch, N.E.; Bose, R.; Kavuri, S.M.; Ma, C.X.; Ellis, M.J. HER2-mutated breast cancer responds to treatment with single-agent neratinib, a second-generation HER2/EGFR tyrosine kinase inhibitor. J. Natl. Compr. Canc. Netw., 2015, *13*(9), 1061-1064.

[126] Jani, J.P.; Finn, R.S.; Campbell, M.; Coleman, K.G.; Connell, R.D.; Currier, N.; Emerson, E.O.; Floyd, E.; Harriman, S.; Kath, J.C. Discovery and pharmacologic characterization of CP-724,714, a selective ErbB2 tyrosine kinase inhibitor. *Cancer Res.*, **2007**, *67*(20), 9887-9893.

[127] Feng, B.; Xu, J.J.; Bi, Y.-A.; Mireles, R.; Davidson, R.; Duignan, D.B.; Campbell, S.; Kostrubsky, V.E.; Dunn, M.C.; Smith, A.R. Role of hepatic transporters in the disposition and hepatotoxicity of a HER2 tyrosine kinase inhibitor CP-724,714. *Toxicol. Sci.*, **2009**, *108*(2), 492-500.

[128] Slichenmyer, W.J.; Elliott, W.L.; Fry, D.W. *CI-1033, a pan-erbB tyrosine kinase inhibitor*. *Semin. Oncol.,* **2001**, *28*, 80-85.

[129] Smaill, J.B.; Showalter, H.H.; Zhou, H.; Bridges, A.J.; McNamara, D.J.; Fry, D.W.; Nelson, J.M.; Sherwood, V.; Vincent, P.W.; Roberts, B.J. Tyrosine kinase inhibitors. 18. 6-Substituted 4-anilinoquinazolines and 4-anilinopyrido [3, 4-d] pyrimidines as soluble, irreversible inhibitors of the epidermal growth factor receptor. *J. Med. Chem.,* **2001**, *44*(3), 429-440.

[130] Rixe, O.; Franco, S.X.; Yardley, D.A.; Johnston, S.R.; Martin, M.; Arun, B.K.; Letrent, S.P.; Rugo, H.S. A randomized, phase II, dose-finding study of the pan-ErbB receptor tyrosine-kinase inhibitor CI-1033 in patients with pretreated metastatic breast cancer. *Cancer Chemother. Pharmacol.*, **2009**, *64*(6), 1139-1148.

[131] Cai, X.; Zhai, H.-X.; Wang, J.; Forrester, J.; Qu, H.; Yin, L.; Lai, C.-J.; Bao, R.; Qian, C. Discovery of 7-(4-(3-ethynylphenylamino)-7-methoxyquinazolin-6-yloxy)-N-hydroxyheptanamide (CUDC-101) as a potent multi-acting HDAC, EGFR, and HER2 inhibitor for the treatment of cancer. *J. Med. Chem.*, **2010**, *53*(5), 2000-2009.

[132] Lai, C.-J.; Bao, R.; Tao, X.; Wang, J.; Atoyan, R.; Qu, H.; Wang, D.-G.; Yin, L.; Samson, M.; Forrester, J. CUDC-101, a multitargeted inhibitor of histone deacetylase, epidermal growth factor receptor, and human epidermal growth factor receptor 2, exerts potent anticancer activity. *Cancer Res.*, **2010**, *70*(9), 3647-3656.

[133] Shimizu, T.; LoRusso, P.M.; Papadopoulos, K.P.; Patnaik, A.; Beeram, M.; Smith, L.S.; Rasco, D.W.; Mays, T.A.; Chambers, G.; Ma, A. Phase I first-in-human study of CUDC-101, a multitargeted inhibitor of HDACs, EGFR, and HER2 in patients with advanced solid tumors. *Clin. Cancer Res.*, **2014**, *20*(19), 5032-5040.

[134] Xie, H.; Lin, L.; Tong, L.; Jiang, Y.; Zheng, M.; Chen, Z.; Jiang, X.; Zhang, X.; Ren, X.; Qu, W. AST1306, a novel irreversible inhibitor of the epidermal growth factor receptor 1 and 2, exhibits antitumor activity both *in vitro* and *in vivo*. *PLoS One,* **2011**, *6*(7), 1-10.

[135] Zhang, J.; Cao, J.; Li, J.; Zhang, Y.; Chen, Z.; Peng, W.; Sun, S.; Zhao, N.; Wang, J.; Zhong, D. A phase I study of AST1306, a novel irreversible EGFR and HER2 kinase inhibitor, in patients with advanced solid tumors. *J. Hematol. Oncol.*, **2014**, *7*(22), 1-11.

[136] Engelman, J.A.; Zejnullahu, K.; Gale, C.-M.; Lifshits, E.; Gonzales, A.J.; Shimamura, T.; Zhao, F.; Vincent, P. W.; Naumov, G. N.; Bradner, J.E. PF00299804, an irreversible pan-ERBB inhibitor, is effective in lung cancer models with EGFR and ERBB2 mutations that are resistant to gefitinib. *Cancer Res.*, **2007**, *67*(24), 11924-11932.

[137] Gonzales, A.J.; Hook, K.E.; Althaus, I.W.; Ellis, P.A.; Trachet, E.; Delaney, A.M.; Harvey, P.J.; Ellis, T.A.; Amato, D.M.; Nelson, J. M. Antitumor activity and pharmacokinetic properties of PF-00299804, a second-generation irreversible pan-erbB receptor tyrosine kinase inhibitor. *Mol. Cancer Ther.*, **2008**, *7*(7), 1880-1889.

[138] Kalous, O.; Conklin, D.; Desai, A.J.; O'Brien, N.A.; Ginther, C.; Anderson, L.; Cohen, D.J.; Britten, C.D.; Taylor, I.; Christensen, J.G. Dacomitinib (PF-00299804), an irreversible Pan-HER inhibitor, inhibits proliferation of HER2-amplified breast cancer cell lines resistant to trastuzumab and lapatinib. *Mol. Cancer Ther.*, **2012**, *11*(9), 1978-1987.

[139] Oh, D.-Y.; Lee, K.-W.; Cho, J.Y.; Kang, W.K.; Rha, S.Y.; Bang, Y.-J. A phase II open-label trial of dacomitinib monotherapy in patients with HER2-positive advanced gastric cancer after failure of at least one prior chemotherapy regimen. *J. Clin. Oncol.*, **2012**, *30*(4), 54-54.

[140] Nagasawa, J.; Mizokami, A.; Koshida, K.; Yoshida, S.; Naito, K.; Namiki, M. Novel HER2 selective tyrosine kinase inhibitor, TAK-165, inhibits bladder, kidney and androgen-independent prostate cancer *in vitro* and *in vivo*. *Int. J. Urol.*, **2006**, *13*(5), 587-592.

[141] Borges, V.F.; Hamilton, E.; Yardley, D.A.; Moulder, S.; Hortobagyi, G.; Walker, L.; Krop, I. In A phase 1b study of ONT-380, an oral HER2-specific inhibitor, combined with ado-trastuzumab emtansine (T-DM1), in HER2+ metastatic breast cancer (MBC). *J Clin. Oncol.,* **2014**, *32*(15), 662-662.

[142] Metzger-Filho, O.; Barry, W.T.; Krop, I.E.; Younger, W.J.; Lawler, E.S.; Winer, E.P.; Lin, N.U. Phase I dose-escalation trial of ONT-380 in combination with trastuzumab in participants with brain metastases from HER2+ breast cancer. *J. Clin. Oncol.,* **2014**, *32*(15), 660.

[143] Cha, M.Y.; Lee, K.O.; Kim, M.; Song, J.Y.; Lee, K.H.; Park, J.; Chae, Y.J.; Kim, Y.H.; Suh, K.H.; Lee, G.S. Antitumor activity of HM781-36B, a highly effective pan-HER inhibitor in erlotinib-resistant NSCLC and other EGFR-dependent cancer models. *Int. J. Cancer,* **2012**, *130*(10), 2445-2454.

[144] Kang, M.H.; Moon, S.U.; Sung, J.H.; Kim, J.W.; Lee, K.W.; Lee, H.S.; Lee, J.S.; Kim, J.H. Antitumor activity of hm781-36b, alone

or in combination with chemotherapeutic agents, in colorectal cancer cells. *Cancer Res. Treat.,* **2016**, *48,* 355-364.

[145] Nam, H.-J.; Kim, H.-P.; Yoon, Y.-K.; Hur, H.-S.; Song, S.-H.; Kim, M.-S.; Lee, G.-S.; Han, S.-W.; Im, S.-A.; Kim, T.-Y. Antitumor activity of HM781-36B, an irreversible Pan-HER inhibitor, alone or in combination with cytotoxic chemotherapeutic agents in gastric cancer. *Cancer Lett.*, **2011**, *302*(2), 155-165.

[146] Im, S.; Kim, J.; Lee, K.; Kim, S.; Oh, D.; Kim, Y.; Han, S.; Kim, T.; Jung, J.; Bang, Y. Abstract P4-13-19: Poziotinib, an oral, irreversible pan-HER inhibitor, demonstrates promising clinical activity in metastatic HER2 positive breast cancer patients. *Cancer Res.*, **2016**, *76*(4), 4-13.

[147] Park, Y.; Jung, K.; Sohn, J.; Lee, K.; Lee, K.; Kim, J.; Kim, J.; Jung, J.; Han, H.; Park, W. Abstract OT3-01-10: A prospective, open-label, single-arm, multi-center, phase II exploratory study to evaluate the efficacy and safety of poziotinib (NOV120101) in patients with HER2-positive metastatic breast cancer who have received at least two prior HER2-directed regimens. *Cancer Res.,* **2016**, *76*(4), 3-10.

[148] Kim, J.; Im, S.; Lee, K.; Kim, J.; Han, S.; Kim, T.; Choi, I.; Oh, D.; Lee, N.; Song, C. 664p Phase IIa study to evaluate the biological activity of aslan001 in her-1/2 co-expressing or HER-2 amplified advanced gastric cancer. *Ann. Oncol.*, **2014**, *25*(4), 1-10.

[149] Xu, B.; Ma, F.; Chen, S.; Li, Q.; Yang, F.; Zhang, Y.; Chen, X.; Zhong, D.; Zhang, G. A phase I study for tolerability, safety, and pharmacokinetics of pyrotinib, a novel irreversible HER2 and EGFR inhibitor, in Chinese patients with HER2+ metastatic breast cancer. *J. Clin. Oncol.*, **2015**, *33*(15), 11596.

[150] Wong, T.; Ayers, M.; Emanuel, S.; Fargnoli, J.; Harbison, C.; Lee, F.; Oppenheimer, S.; Yu, C.; Krishnan, B.; Zhang, H. Inhibition of EGFR/HER2 signaling in tumor cells and VEGFR2 activity in tumor endothelium contribute to the preclinical anti-tumor activity of BMS-690514. *Cancer Res.,* **2007**, *67*(9), 4007.

[151] Silva, S.; Jackson, V.; Guise, C.; Abbattista, M.; Bull, M.; Grey, A.; Anderson, R.; Ashoorzadeh, A.; Hart, C.; Pearce, T. Abstract A67: Preclinical efficacy of tarloxotinib bromide (TH-4000), a hypoxia-activated EGFR/HER2 inhibitor: rationale for clinical evaluation in EGFR mutant, T790M-negative NSCLC following progression on EGFR-TKI therapy. *Mol. Cancer Ther.,* **2015**, *14*(2), 67.

[152] Nakayama, A.; Takagi, S.; Yusa, T.; Yaguchi, M.; Hayashi, A.; Tamura, T.; Kawakita, Y.; Ishikawa, T.; Ohta, Y. Antitumor activity of TAK-285, an investigational, non-Pgp substrate HER2/EGFR kinase inhibitor, in cultured tumor cells, mouse and rat xenograft tumors, and in an HER2-positive brain metastasis model. *J. Cancer*, **2013**, *4*(7), 557-565.

[153] Erdő, F.; Gordon, J.; Wu, J.-T.; Sziráki, I. Verification of brain penetration of the unbound fraction of a novel HER2/EGFR dual kinase inhibitor (TAK-285) by microdialysis in rats. *Brain Res. Bull,* **2012**, *87*(4), 413-419.

[154] LoRusso, P.; Venkatakrishnan, K.; Chiorean, E. G.; Noe, D.; Wu, J.-T.; Sankoh, S.; Corvez, M.; Sausville, E.A. Phase 1 dose-escalation, pharmacokinetic, and cerebrospinal fluid distribution study of TAK-285, an investigational inhibitor of EGFR and HER2. *Invest. New Drugs,* **2014**, *32*(1), 160-170.

[155] Traxler, P.; Allegrini, P.R.; Brandt, R.; Brueggen, J.; Cozens, R.; Fabbro, D.; Grosios, K.; Lane, H.A.; McSheehy, P.; Mestan, J. AEE788. *Cancer Res.*, **2004**, *64*(14), 4931-4941.

[156] Gavai, A.V.; Fink, B.E.; Fairfax, D.J.; Martin, G.S.; Rossiter, L.M.; Holst, C.L.; Kim, S.-H.; Leavitt, K.J.; Mastalerz, H.; Han, W.-C. Discovery and preclinical evaluation of [4-[[1-(3-fluorophenyl) methyl]-1 H-indazol-5-ylamino]-5-methylpyrrolo [2, 1-f][1, 2, 4] triazin-6-yl] carbamic Acid,(3 S)-3-Morpholinylmethyl Ester (BMS-599626), a selective and orally efficacious inhibitor of human epidermal growth factor receptor 1 and 2 kinases. *J. Med. Chem.*, **2009**, *52*(21), 6527-6530.

[157] Wong, T.W.; Lee, F.Y.; Yu, C.; Luo, F.R.; Oppenheimer, S.; Zhang, H.; Smykla, R.A.; Mastalerz, H.; Fink, B.E.; Hunt, J.T. Preclinical antitumor activity of BMS-599626, a pan-HER kinase inhibitor that inhibits HER1/HER2 homodimer and heterodimer signaling. *Clin. Cancer Res.*, **2006**, *12*(20), 6186-6193.

[158] Garland, L.; Pegram, M.; Song, S.; Mendelson, D.; Parker, K.; Martell, R.; Gordon, M. Phase I study of BMS-599626, an oral pan-HER tyrosine kinase inhibitor, in patients with advanced solid tumors. *J. Clin. Oncol.*, **2005**, *23*(16), 3152-3152.

[159] Tanaka, H.; Hirata, M.; Shinonome, S.; Wada, T.; Iguchi, M.; Dohi, K.; Inoue, M.; Ishioka, Y.; Hojo, K.; Yamada, T. Preclinical antitumor activity of S-222611, an oral reversible tyrosine kinase inhibitor of epidermal growth factor receptor and human epidermal growth factor receptor 2. *Cancer Sci.*, **2014**, *105*(8), 1040-1048.

[160] Hirata, M.; Tanaka, H.; Dohi, K.; Iguchi, M.; Wada, T.; Matsuo, K.; Kume, M.; Omori, N.; Tada, Y.; Orita, S. Abstract# 1761: S-222611, a potent, orally active small molecule inhibitor of EGFR and HER2: *in vitro* kinase inhibition and antitumor activity. *Cancer Res.,* **2009**, *69*, 1761-1761.

[161] Deva, S.; Baird, R.D.; Cresti, N.; Garcia-Corbacho, J.; Hogarth, L.; Frenkel, E.P.; Kawaguchi, K.; Arimura, A.; Donaldson, K.; Posner, J. Phase I expansion of S-222611, a reversible inhibitor of EGFR and HER2, in advanced solid tumors, including patients with brain metastases. *J. Clin. Oncol.,* **2015**, *33*(15), 2511.

[162] MedKoo Biosciences, Inc. www.medkoo.com/products/6917.

[163] National Cancer Institute. NCI- Drug Dictionary. www.cancer.gov/publications/dictionaries/cancer-drug?cdrid=772247.