Epidermal Growth Factor Receptor Irreversible Inhibitors: Chemical Exploration of the Cysteine-Trap Portion

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Abstract: Covalent EGFR irreversible inhibitors showed promising potential for the treatment of gefitinib-resistant tumors and for imaging purposes. They contain a cysteine-reactive portion forming a covalent bond with the protein. Irreversible kinase inhibitors have been advanced to clinical studies, mostly characterized by an acrylamide or butynamide warhead. However, the clinical usefulness of these compounds has been hampered by resistances, toxicity and pharmacokinetic problems. Investigation on the structure-activity and structure-reactivity relationships may provide useful information for compounds with improved selectivity and pharmacokinetic properties. This review focuses on the exploration of the cysteine-trap portions able to irreversibly inhibit EGFR and other erbB receptors.

Keywords: Antitumor, covalent inhibition, cysteine-trap, EGFR, erbB receptors, irreversible inhibitors, NSCLC.

1. INTRODUCTION

The epidermal growth factor receptor (EGFR) is a transmembrane receptor-tyrosine kinase belonging to the erbB family that is abnormally activated in several epithelial tumors. The erbB family includes four related members: erbB1/EGFR, erbB2/HER2, erbB3/HER3, and erbB4/HER4 [1, 2]. These receptors are composed by an extracellular ligand-binding domain, a transmembrane domain, and an intracellular domain with tyrosine kinase activity. Upon ligand binding, EGFR undergoes homo- or hetero-dimerization and autophosphorylation of specific tyrosine residues within the intracellular domain. The autophosphorylated receptor activates a variety of intracellular transduction pathways that promote cell growth, proliferation, differentiation and migration [3, 4]. Deregulation of EGFR signaling has been observed in many human cancers, including lung, head and neck, colorectal, ovarian, breast and bladder cancers [5, 6], and it has been associated with more aggressive disease and poorer clinical outcome [7]. Therefore, inhibitors targeting the EGFR have been extensively investigated as antitumor agents.

Hyperactivation of EGFR can be produced by gene amplification, receptor overexpression, activating mutations, overexpression of receptor ligands and/or loss of regulatory controls [8]. Several oncogenic mutations in EGFR have been related to the development of cancer, particularly non-small cell lung cancer (NSCLC) [9, 10]. Activating mutations of the *EGFR* gene are found in the first four exons (18 through 21) of the TK domain: deletions in exon 19 (del19), a substitution mutation in the exon 21 (L858R), and less common mutations (*e.g.* G719S) enable constitutive activation of the kinase function, stabilizing the active conformation of the kinase domain in the absence of ligand-induced stimulation [11, 12]. Two selective EGFR inhibitors, gefitinib [13] (**1**, Iressa, Astra Zeneca, Fig. (**1**)), and erlotinib [14] (**2**, Tarceva, OSI Pharmaceuticals, Fig. (**1**)), have higher potency against these mutant kinases than the wild-type enzyme [15] and they are approved for the treatment of NSCLC in patients having the activating mutations of EGFR. These tyrosine kinase inhibitors, belonging to the chemical class of 4-anilinoquinazolines, compete with ATP in a reversible manner, binding to the kinase domain of the target through weak interactions (hydrogen-bonds, van der Waals and hydrophobic interactions). Although gefitinib (**1**) is effective in the treatment of NSCLC bearing the oncogenic mutations of EGFR, accumulating clinical experience indicates that most patients develop resistance after repeated treatments [10]. In approximately half of NSCLC cases that show an initial response to reversible EGFR tyrosine kinase inhibitors and that subsequently progress, resistance is associated with the emergence of a single amino acid substitution in the catalytic domain of EGFR: conversion of the gatekeeper residue threonine 790 with methionine (T790M) [10, 16, 17]. T790M substitution appears to increase the affinity of EGFR for ATP and to eliminate the hypersensitivity to tyrosine kinase inhibitors conferred by the L858R mutation [18]. Additional mechanisms of resistance to reversible EGFR inhibitors have been described, such as the activation of alternative tyrosine kinase receptors (IGF-1R), amplification of the MET gene and constitutive activation of signaling pathways downstream of EGFR [19, 20].

Cancers that become resistant to kinase inhibitors through a secondary mutation are still likely to be dependent

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on the activated kinase for their growth and survival. This has prompted the development of second-generation irreversible EGFR inhibitors that overcome resistance [21-23] by covalent alkylation of a cysteine residue (Cys797) close to the ATP binding site of EGFR [24, 25]. Irreversible kinase inhibitors possess several advantages over conventional reversible ATP-competitive inhibitors. Considering that the targeted cysteine residue is conserved within the erbB-family kinase domains (Cys797 in EGFR, Cys805 in erbB2, and Cys803 in erbB4), and that only a limited group of kinases has a cysteine at the corresponding position [26], irreversible inhibitors are rather selective for erbB-family tyrosine kinases. In addition, covalent bond formation can circumvent competition with ATP, which under physiological conditions has intracellular concentration in the millimolar range. Furthermore, an irreversible inhibitor does not need prolonged circulating levels in blood to achieve the desired biological effect: once the target enzyme is deactivated by covalent bond formation, the biological effect can persist even after drug disappearance from circulation. As a result, the duration of action of such a drug depends on the rate of enzyme turnover rather than on drug pharmacokinetics.

A long-lasting effect on tyrosine kinase inhibition has also been described for lapatinib [27] (**3**, Tykerb, GSK, Fig. (**1**)), approved for the treatment of advanced breast cancer. Lapatinib (**3**) is a potent dual EGFR and erbB2 inhibitor that exhibited reversible, non-covalent inhibition of tyrosine kinase characterized by very slow off-rate from the purified enzymes and prolonged down-regulation of the receptor in tumor cells. Crystal structure of EGFR bound to lapatinib (**3**) revealed the kinase in an inactive-like conformation, thus reducing the rate of inhibitor dissociation and allowing for prolonged effects in biological systems [27].

EGFR covalent irreversible inhibitors are characterized by a heterocyclic, generally bicyclic, core structure (driving portion) carrying at a proper position an electrophilic functionality (warhead) able to covalently interact with the conserved, solvent-exposed cysteine residue present in the target protein. A number of irreversible kinase inhibitors have been submitted to clinical studies, Fig. (**1**); their structures are mostly characterized by a 4-anilinoquinazoline or a 4-anilino-3-cyanoquinoline driving portion and by an acrylamide, a substituted acrylamide or a butynamide warhead. These inhibitors are currently undergoing clinical testing in patients that initially responded to erlotinib and gefitinib and subsequently relapsed [28]. However, in the past decade several driver portion-warhead combinations have been explored to identify new drug-like leads for the development of irreversible erbB tyrosine kinase inhibitors

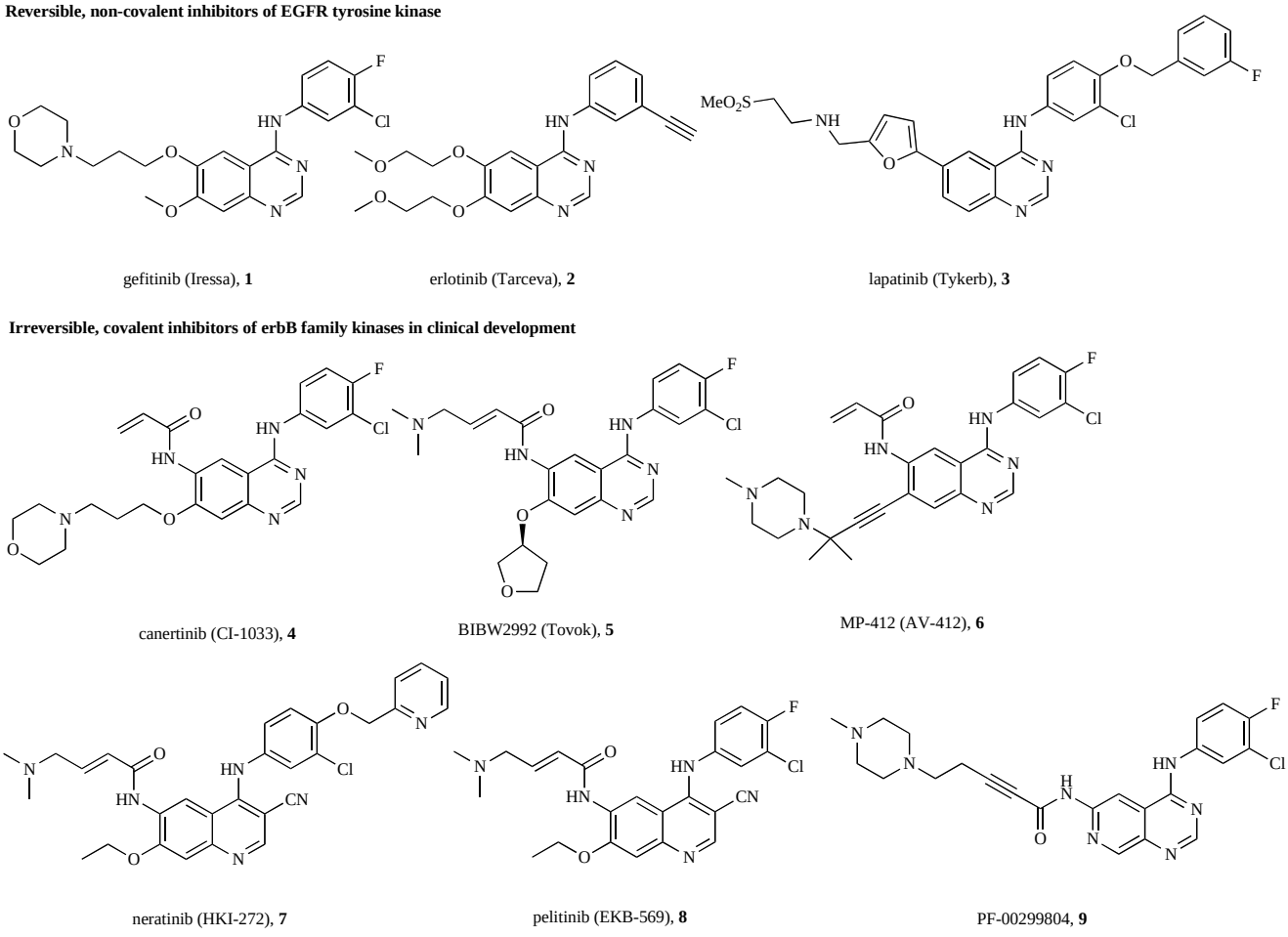


Fig. (1). EGFR inhibitors in clinical use or development.

as potential candidates for cancer therapy. In principle the driving portion should assure both high target affinity, by containing the structural elements required for interaction with the ATP-binding site of EGFR, and target selectivity, by carrying the electrophilic functionality at the proper position, compatible with the formation of the critical bond. The intrinsic reactivity of cysteine-reactive groups should be sufficiently low to avoid indiscriminate reaction with non-target-related proteins that could lead to toxic effects. Therefore, it is important that these covalent binding agents have (i) low reactivity, (ii) a reasonably good fit at the active site of the target, and (iii) the reactive centers held in close proximity and oriented in the proper manner for a covalent interaction to ensue. This review focuses on the chemical exploration of the cysteine-reactive portions of irreversible EGFR inhibitors. The electrophilic warheads within this review contain chemical groups ranging from those potentially reactive toward generic nucleophiles, such as in the case of acrylamides or butynamides, to less-reactive functionalities, that only react upon target binding, such as alkynylthienopyrimidines. When known, we include a description of the proposed reaction mechanism, crystal structures, as well as intrinsic reactivity studies, and drugs or compounds in advanced clinical development within each class.

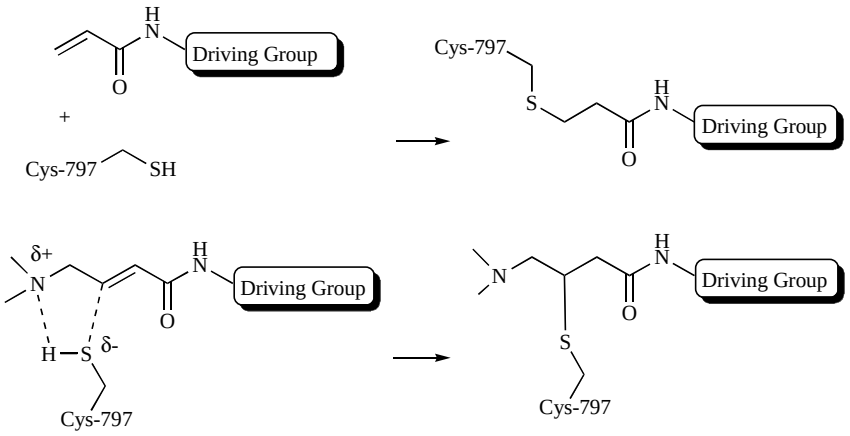
2. ACRYLAMIDES

Acrylamides are Michael-acceptor compounds that can react with soft nucleophiles, such as thiols, giving conjugate-addition products (Scheme **1**). Potent and relatively selective irreversible EGFR inhibitors can be obtained by introducing an acrylamide fragment on the heterocyclic structure of known reversible EGFR binders. This strategy has led to a number of effective covalent inhibitors, which have been advanced into clinical trials. These include the 4-anilinoquinazolines Canertinib [29] (**4**, CI-1033, Pfizer, Fig. (**1**)), BIBW2992 [30] (**5**, Tovok, Boehringer Ingelheim, Fig. (**1**)) and MP-412 [31] (**6**, AV-412, Aveo Pharmaceuticals, Fig. (**1**)); and the 4-anilino-3-cyanoquinolines neratinib [32] (**7**, HKI-272, Wyeth, Fig. (**1**)), and pelitinib [33] (**8**, EKB-569, Wyeth, Fig. (**1**)).

Alkylation of the thiol group of Cys797 in the ATP binding pocket of EGFR was demonstrated for the 6-acrylamido-4-anilinoquinazoline PD168393, compound (**10**) in Fig. (**2**), by mass spectrometry and site-directed mutagenesis [24, 25]. Co-crystallization of **10** within the kinase domain of human EGFR [25], Fig. (**3**), showed that the inhibitor is covalently bound to Cys797 and adopts an accommodation similar to that previously observed for several reversible quinazoline inhibitors in complex with kinases [12, 27, 34]. The covalent bond is formed between the β-carbon atom of the acrylamide Michael acceptor on **10** and the γ-sulfur atom of Cys797. Moreover, N1 and N3 on the quinazoline driving portion are involved in two crucial hydrogen bonds, one with the backbone nitrogen of Met793 and the other with the side chain of Thr790, through a conserved water molecule. The 4-aniline substituent points toward the hydrophobic pocket beyond the gatekeeper Thr790 [25], as shown in Fig. (**3**). Crystal structures of the T790M mutant with the irreversible EGFR inhibitor neratinib, which belongs to the class of 3-cyanoquinolines, revealed that the thiol group of Cys797 is covalently bound to the inhibitor, which has its bicyclic scaffold positioned in the same way as the quinazoline one of **10** [12]. On the other hand, there are differences between the two protein-ligand adducts: the methionine side chain of the mutated kinase cannot participate in a hydrogen-bond network, although it does not impede the accommodation of the cyano group of neratinib, which is considered to mimic the bridging water molecule of EGFR-quinazoline complexes. Moreover, the lapatinib-like side chain of neratinib obviously affects the interaction with the enzyme of the aniline moiety, and the ex-acrylamide portions adopt, in the two protein-inhibitor adducts, different conformations [18, 25].

Kinetic studies on the 6-acrylamido derivative (**10**) and the 7-acrylamido isomer PD160678, compound (**11**) in Fig. (**2**), revealed that the 7-substituted inhibitor, despite still being irreversible, reacts more slowly with the enzyme [24]. Thus, even if there is some tolerance to the spatial arrangement of the Michael-acceptor portion, its position on the quinazoline nucleus influences the efficiency of the addition, and could be a discriminating issue for irreversible inhibition.

With the 6-position generally (but not always) preferred as the attachment point for the electrophilic warhead,



Scheme 1. Reaction mechanism of acrylamides toward Cys797.

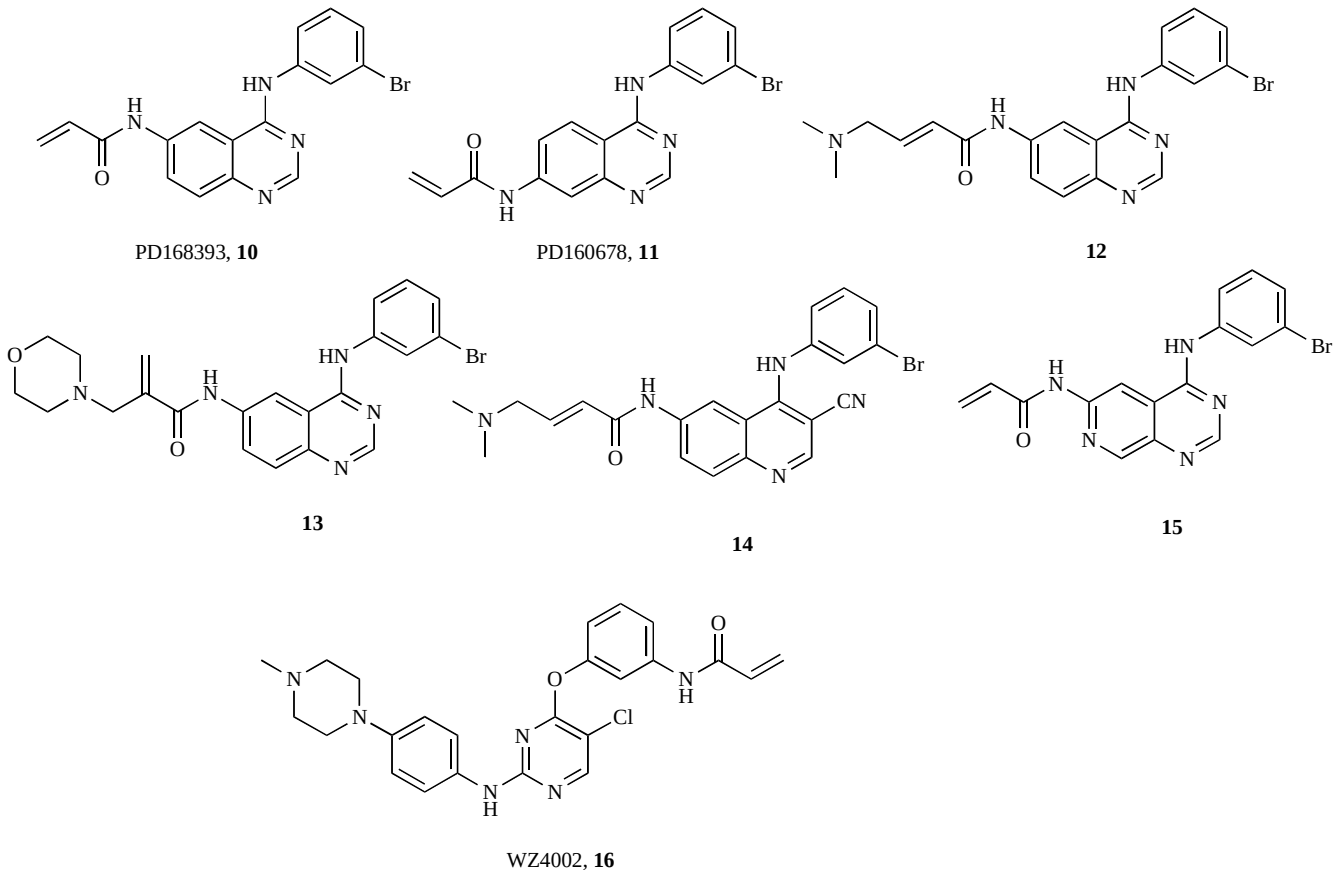


Fig. (2). Representative acrylamide-based irreversible EGFR inhibitors.

structure-activity studies have been performed on the acrylamide group, to tune its reactivity or to increase the overall solubility of the compound [35-39]. Minimal substitution at the acrylamide nitrogen was permitted, with only a methyl group being tolerated. Alkyl substituents on the α- or β-carbons of the acrylamide group were also detrimental for irreversible inhibition [35]. On the other hand, a soluble side chain pendent off the α- and β-carbons of the Michael acceptor through a methylene spacer (**12** and **13**, Fig. (**2**)) is well tolerated, providing irreversible inhibitors of EGFR with improved pharmacokinetic properties [36]. Such groups have been inserted at the 6- and/or 7- positions, since both these positions point out of the ATP-binding pocket, reaching a solvent-accessible area [34]. It has been proposed that when the water-solubilizing group is a dialkylamino group, located on the Michael acceptor at the 6 position, it can also serve as an intramolecular catalyst for the Michael addition of the sulfhydryl group of Cys797 *via* a catalytic mechanism (Scheme **1**) [36]. On the other hand, since the dialkylamino group exists predominantly in its protonated form under physiological conditions, it could simply exert an inductive effect, accelerating the Michael addition [36]. In any case, the attachment of a dialkylaminomethyl group to the acrylamide fragment led to the development of the clinical candidates BIBW2992 (**5**) [30], neratinib (**7**) [32, 40] and pelitinib (**8**) [33, 41], reported in Fig. (**1**). A basic side chain can also be introduced at a position different from that of the Michael-acceptor group, yet giving soluble and irreversible inhibitors, suitable for clinical development. These include MP-412 (**6**) [31], Fig. (**1**), and canertinib (**4**) [37], Fig. (**1**), a 7-morpholinopropoxy-6-acrylamide quinazoline derivative that shows high aqueous solubility and good oral bioavailability as dihydrochloride salt. Considering that these compounds target a cysteine residue conserved within the kinase domains in the erbB-family, the presence of the Michael acceptor generally confers EGFR/erbB2 inhibitor activity and in some cases pan-erbB activity. Additionally, appropriate decorations on the recognition portion can improve erbB2 affinity, as in the case of neratinib, a dual EGFR/erbB2 irreversible inhibitor, that contains a 4-anilino side chain similar to that of lapatinib (**3**) [27].

Recent clinical studies on the irreversible EGFR tyrosine kinase inhibitors BIBW2992 (**5**) [42] and neratinib (**7**) [43] revealed that the most frequent adverse effects observed during treatments were gastrointestinal toxicity (*e.g.* diarrhea, nausea and vomiting), fatigue, and skin rash. This pattern of toxicity was in line with toxicities associated to first-generation reversible kinase inhibitors gefitinib (**1**), erlotinib (**2**) and lapatinib (**3**) [44-46], which is encouraging particularly for BIBW2992, if compared to the increased efficacy on gefitinib-resistant tumors [42].

The intrinsic reactivity of the acrylamide warhead could also be the cause of metabolic degradation and lack of target selectivity, giving rise to potentially increased and

unexpected toxicity. For this reason, reactivity studies have been performed in the presence of bionucleophiles, generally represented by glutathione. Covalent EGFR inhibitors belonging to the acrylamido class exhibited relatively low reaction rates with glutathione, showing that, even if non-specific reactions with electrophile off-targets are possible, the acrylamido fragment is sufficiently stable to be employed for specific kinase inhibition in biological environments [36, 41].

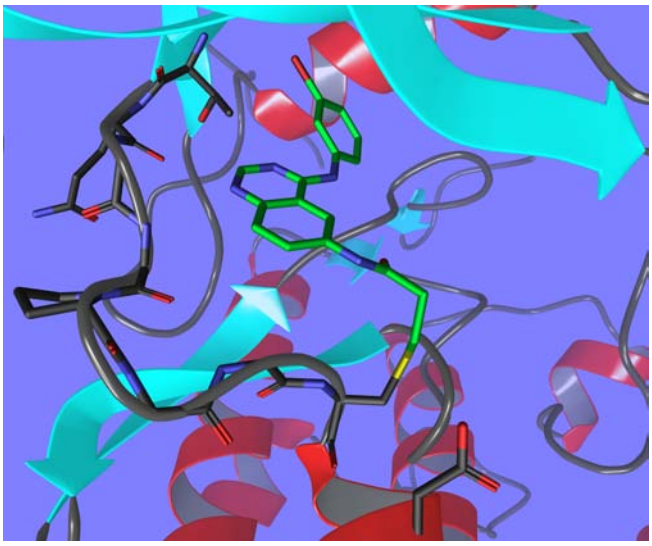


Fig. (3). Co-crystal structure of the 6-acrylamide-4-aniloquinazoline PD168393 (**10**) within the catalytic site of EGFR. Adapted from Ref. [25].

Parallel to the exploration of the acrylamide warhead, several driving portions carrying the structural elements for interaction with the ATP-binding pocket of EGFR have been explored, in order to optimize the fitting at the receptor active site and to improve potency and selectivity. Besides the 4-anilinoquinazoline scaffold, the acrylamide warhead has also been inserted at the 6 position of other heterocyclic rings, such as 4-anilino-3-cyanoquinolines [40, 41] (**14**, Fig. (**2**)), 4-anilinopyrido[*d*]pyrimidines [35, 37, 47] (**15**, Fig. (**2**)), and 4-anilino-3-cyano[1,7] and [1,8]naphtyridine [48]. Recently, a novel chemotype, an acrylamide-substituted 2,4-dianilino-5-chloropyrimidine (WZ4002 (**16**), Fig. (**2**)), has been reported to inhibit T790M mutant EGFR [49]. Interestingly, this agent was 100-fold more potent against EGFR T790M than wild-type EGFR. Co-crystallization of **16** in complex with EGFR L858R/T790M revealed that, while forming the expected covalent bond with Cys797, the anilinopyrimidine scaffold has an intrinsically better fit with the hydrophobic ATP-binding site of the mutated enzyme, compared to 4-anilinoquinazoline-based irreversible inhibitors. This mutant-selective profile may turn out to be useful in clinical settings, enabling greater therapeutic windows due to inhibition of mutant EGFR while minimizing toxicities, mediated by the inhibition of wild-type EGFR.

3. BUTYNAMIDES

Butynamides provide irreversible inhibitors when inserted on a suitable scaffold, able to recognize the EGFR kinase domain [39, 50]. The alkynamide warhead can react with bionucleophiles, including thiols of cysteines, giving Michael-type addition product (Scheme **2**). The reference compound of this class CL-387785 (**17**, Fig. (**4**)) was demonstrated to bind covalently to EGFR by incubating [^{14}C]-**17** with a membrane preparation derived from a cell line overexpressing EGFR. The radiolabeled 170-kD protein was detected, and such labeling was competed by the unlabeled compound (**17**) [50]. Molecular modeling studies supported this experimental finding, showing that the β-carbon atom of the Michael acceptor functional group of compound (**17**) can be accommodated at bonding distance to the sulfhydryl group of Cys797. In addition, the model suggested that the amino group of a lysine residue (Lys728) can be located close enough to this sulfhydryl group to serve as a basic catalyst for the Michael addition reaction (Scheme **2**) [50].

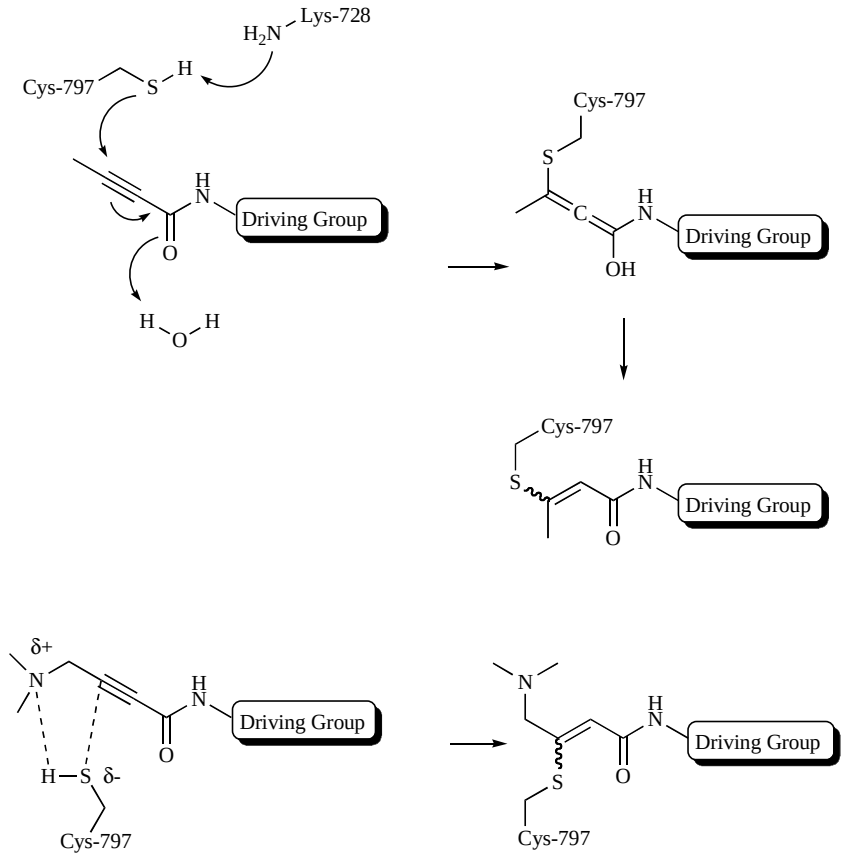
Similarly to what had been observed for the series of unsubstituted acrylamides, butynamide compounds exhibit poor bioavailability after oral administration, primarily because of their low solubility. To improve the pharmacokinetic properties of these compounds, water-solubilizing groups were incorporated on the Michael acceptor attached at the 6 position on the heterocyclic nucleus [36]. The improved water solubility resulted in butynamide compounds, such as compound (**18**), reported in Fig. (**4**), with enhanced biological properties [36, 41]. Reactivity studies with glutathione showed that the presence of the amino group increases the reactivity of butynamide derivatives, thus supporting the hypotheses of intramolecular catalysis for the Michael addition (Scheme **2**) and/or increased electrophilicity of the multiple bond. Nevertheless, alkynamides proved generally less reactive with respect to the corresponding acrylamides [36].

The alkynamide warhead has also been inserted on different receptor-recognition scaffolds, such as 4-anilino-3-cyanoquinoline [36, 41] (**19**, Fig. (**4**)), 4-anilinopyrido[*d*] pyrimidine [51] (**20**, Fig. (**4**)), and 4-anilino-3-cyano[1,7] naphtyridine [48]. Among the compounds under clinical investigation, the pyrido[3,4-*d*]pyrimidine PF-00299804 (**9**, Pfizer, Fig. (**1**)) has emerged as a promising one, with an optimal pharmacological profile characterized by pan-erbB activity and low toxicity [51].

4. VINYLSUFONAMIDES

Vinylsulfonamides have been employed in several cases as alternative Michael acceptors. In fact, compounds carrying this warhead are able to irreversibly inhibit EGFR [35, 39]. The reaction mechanism of vinylsulfonamides with thiols is similar to that of acrylamides, with the formation of the Michael-addition product (Scheme **3**).

Although vinylsulfonamides (**21**) and (**22**) (Fig. (**5**)) irreversibly inhibit EGFR both in enzyme and cellular assays, subsequent *in vivo* experiments showed that compound (**22**) undergoes chemical degradation in biological systems [35]. Similar results have also been obtained from vinylsulfonamide-containing 3-cyano-quinolines, such as **23** [40] in Fig. (**5**), so that this warhead was no further developed. Within the same work,



Scheme 2. Reaction mechanism of butynamides toward Cys797.

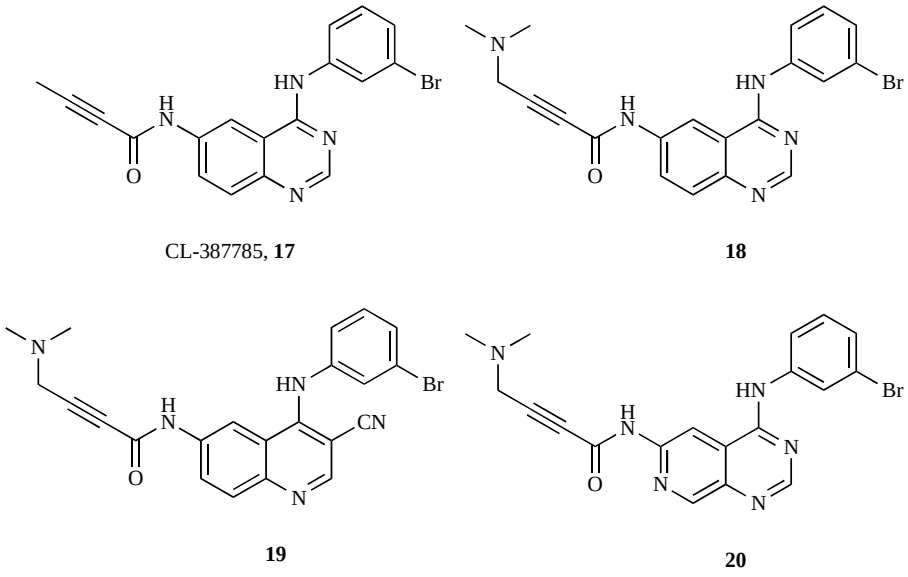
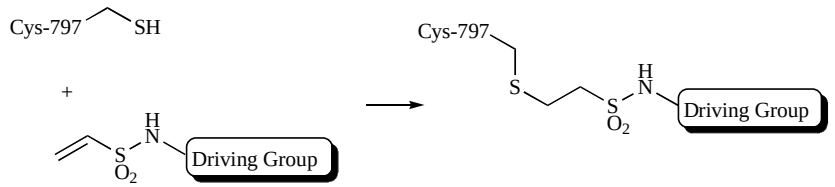


Fig. (4). Representative butynamide-based irreversible EGFR inhibitors.

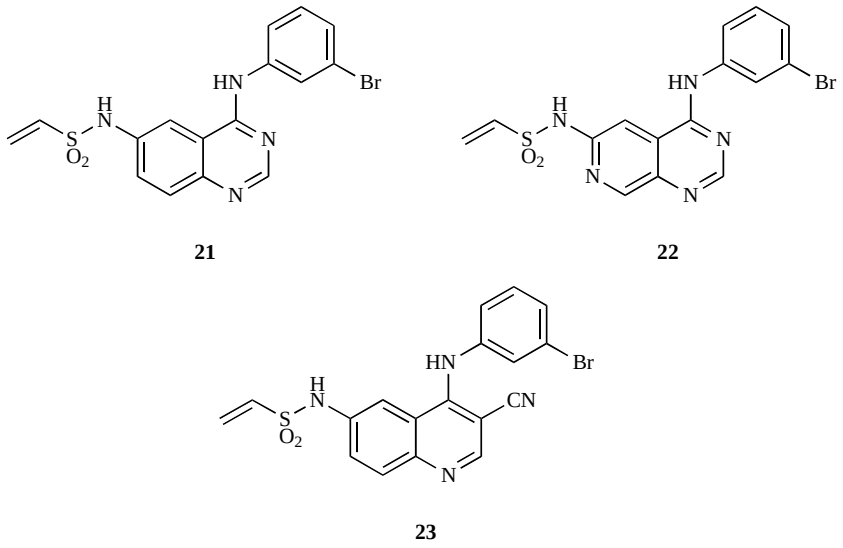
vinylsulfones and vinylsulfines were also considered as alternative cysteine-reactive species, but the corresponding compounds resulted poorly active [35].

5. α-SUBSTITUTED ACETAMIDES

Modulation of the cysteine-reactive portion in EGFR inhibitors led to the identification of α-substituted acetamides, Fig. (**6**), as viable warheads able to irreversibly inhibit the enzyme [52, 53]. Structure-activity relationship studies explored 4-anilinoquinazoline derivatives having, at position 6, a α-methoxyacetamide [52] (**24**), a α-chloroacetamide [52] (**25**), or a α-phenoxyacetamide [53] (**26** and **27**) fragment.

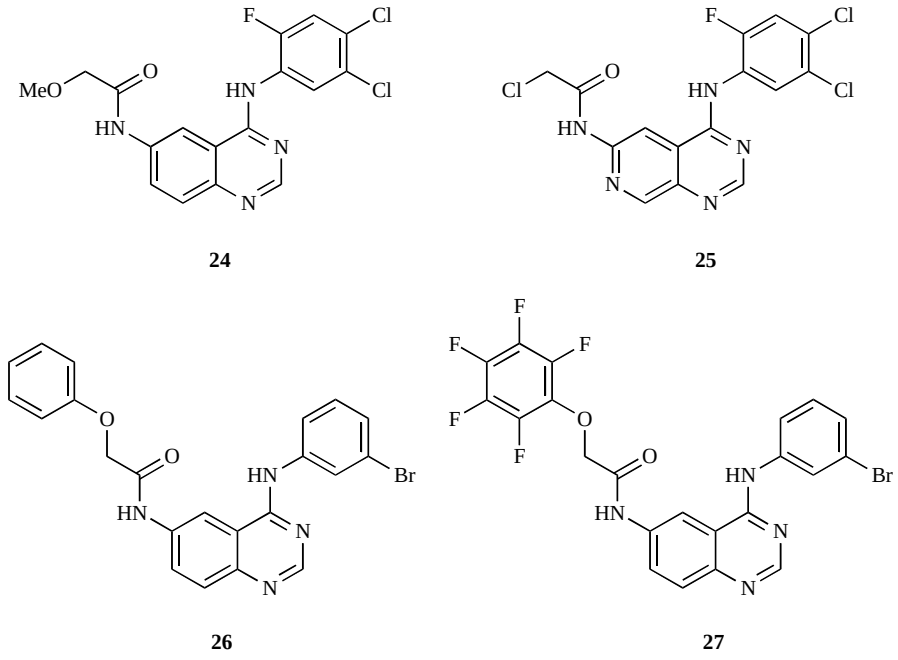


Scheme 3. Proposed reaction mechanism of vinylsulfonamides toward Cys797.



21 **22** **23**

Fig. (5). Representative vinylsulfonamide-based irreversible EGFR inhibitors.

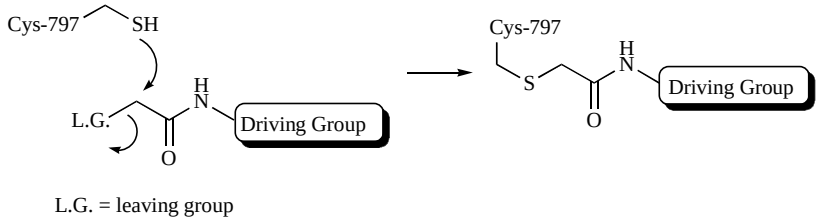


24 **25** **26** **27**

Fig. (6). Representative acetamide-based irreversible EGFR inhibitors.

In has been proposed [53] that the reaction mechanism of activated acetamides toward nucleophiles consists in a nucleophilic substitution with *in situ* release of a leaving group (Scheme **4**). Thus, the α-carbon of compound (**24**-**27**) is supposed to form a stable covalent bond with Cys797 sulfur atom at the EGFR active site.

To demonstrate the irreversible effect of the compounds on EGFR tyrosine kinase, inhibition of EGFR autophosphorylation in intact cells was measured either immediately after or 8 h after removal of the inhibitor from the reaction medium [24]. Substituted acetamides proved



Scheme 4. Proposed reaction mechanism of activated acetamides toward Cys797.

effective in irreversibly block EGFR activity, showing persisting inhibition 8 h after wash-out.

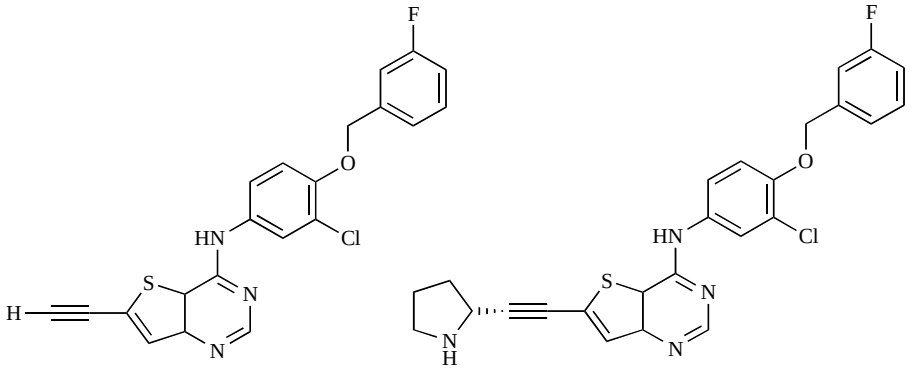
Intrinsic reactivity studies on substituted acetamides in the presence of the sulfhydryl-containing tripeptide glutathione have also been performed. Results were compared with those obtained with the acrylamide derivative (**10**). Compound (**10**) was found to be the most chemically reactive toward the nucleophilic attack performed by glutathione. The chloroacetamide (**25**) was slightly less reactive, while methoxyacetamide (**24**) and phenoxyacetamide (**26**) were found to be far more chemically stable toward glutathione. The weak reactivity of **24** resulted in weak inhibition of EGFR and poor irreversible effect. On the other hand, phenoxyacetamides (**26**) and (**27**) proved the best balance, within the series, between intrinsic reactivity and inhibitory effect, showing irreversible EGFR inhibition and very low or absent reactivity toward glutathione in the tested conditions [53]. Interestingly, beyond its interest as a warhead, phenoxyacetamide derivatives could also be exploited to design and optimize leaving groups that may be pharmacologically active in a multi-target approach [54, 55].

6. ALKYNYL THIENOPIRIMIDINES

6-Alkynyl-thieno[3,2-*d*]pyrimidines have been recently reported as potent and effective EGFR/erbB2/erbB4 inhibitors. Although this class of inhibitors does not possess an evident Michael-acceptor fragment, conjugation of the ethynyl group with the bicyclic scaffold allows the addition of a thiol group to occur in biological conditions. In fact, co-crystallization of compound (**28**), Fig. (**7**), within the catalytic domain of erbB4, a close homolog of EGFR and erbB2, revealed the existence of a covalent bond between the terminal ethynyl carbon and the sulfur atom of Cys803 (corresponding to Cys797 in EGFR) [56]. The X-ray crystal structure captured the enzyme in an inactive conformation, similar to that of EGFR complexed with lapatinib (**3**), which is obviously related to the presence of a lapatinib-like aniline moiety in the inhibitor [57]. In fact, while the pyrimidine N1 atom of the inhibitor forms a hydrogen bond with a methionine residue in the hinge region, the aniline substituent occupies the hydrophobic back-pocket in a manner similar to that of lapatinib (**3**) in EGFR [27]. Mass spectrometry investigations showed that a covalent adduct between compound (**28**) and a cysteine residue is formed also in the case of EGFR [56]. As data clearly demonstrated that modification of EGFR occurs at Cys797, alkylation of erbB-family enzymes might be a general phenomenon for this class of inhibitors.

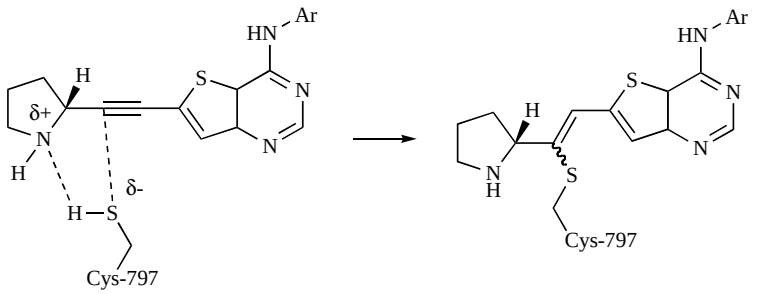
Kinetic experiments relative to the cysteine-alkylation reaction revealed that compounds containing a basic pyrrolidine substituent on the ethynyl terminus (*e.g.* **29**, Fig. (**7**)), show greater reactivity toward EGFR than their unsubstituted analogues, and that the reaction rate can be modulated in a structurally specific manner. Furthermore, the aniline substituent at position 4 on the thienopyrimidine ring was optimized in order to obtain a better fit at the ATP-binding site and to improve kinase selectivity [58].

The proposed reaction mechanism for covalent modification of Cys797 of EGFR by compound (**29**) consists in the initial formation of a non-covalent binding complex with the enzyme. The basic amine could support thiol deprotonation, enabling nucleophilic addition at the terminal ethynyl carbon by the thiolate anion (Scheme **5**). As in the cases previously presented, this mechanism could also be



28 **29**

Fig. (7). Representative alkynyl thienopyrimidine irreversible EGFR/erbB2 inhibitors.



Scheme 5. Proposed reaction mechanism of 6-ethynylthieno[2,3-*d*]pyrimidine with Cys797.

assisted by the presence of a neighboring aspartic acid residue (Asp800) [56] in a way that deserves further investigation.

7. MISCELLANEOUS

In the search of highly specific and non-toxic irreversible EGFR inhibitors, other warheads characterized by weak intrinsic reactivity have been described. 4-Anilino-quinazoline compounds containing an epoxy group at position 6, such as **30** and **31** in Fig. (**8**), irreversibly inhibited EGFR [39, 53]. This class of compounds can undergo a nucleophilic attack on the epoxyde by Cys797, to generate an addition product. As previously observed for both acrylamide and propargylamide derivatives, the introduction of a basic group in the warhead-containing side chain improved inhibitor potency (**31** *vs.* **30**) [53]. The protonated nitrogen of the piperidine ring can form an additional hydrogen bond with the acidic group of Asp800 and this interaction, besides improving the recognition of **31** at the active site, may facilitate the nucleophilic attack at the epoxy ring by Cys797. Intrinsic reactivity of the epoxy warhead toward thiol nucleophiles has been assessed. Notably, no glutathione-adduct formation was detected in aqueous buffered solution for the epoxy derivative (**30**), while the acrylamide (**10**) showed 36% conversion under the same conditions, even if the epoxy group resulted rather unstable in solution [53].

Mixed disulfides, such as disulfide (**32**) [39] and 1,2-dithiolane (**33**) [59] in Fig. (**8**), have also been proposed as thiol traps. In principle, disulfide compounds should be able to covalently bind the cysteine residue of EGFR *via* a thiol-disulphide interchange reaction. These compounds displayed high inhibitory activity on the isolated enzyme, but in cells they were weaker inhibitors, presumably reflecting the difficulty of using sulfide exchange reactions in the cellular environment. A similar reaction mechanism has been proposed for isothiazolinone, benzisothiazolinone and thiadiazole derivatives (**34**, **35** and **36**, respectively, Fig. (**8**)) [53], which can form a disulfide bond with Cys797. In particular, the thiadiazole (**36**) was able to produce a significant, yet partial, irreversible inhibition of EGFR.

Carbamates can form covalent adducts with cysteines by carbamoylation, giving thiocarbamates that can be slowly hydrolyzed to re-establish the enzyme in the active form [60, 61]. Compound (**37**) in Fig. (**8**), characterized by a carbamate warhead linked to the quinazoline driver group through a glycine spacer, behaves as an irreversible inhibitor of EGFR in a modified A431-cellular autophosphorylation assay [24] containing a drug wash-out protocol [53]. Also the cyanoacetamide group has been explored as a warhead, and derivative (**38**), Fig. (**8**), inhibited EGFR autophosphorylation showing a partially irreversible effect. In fact, nitriles are known to form, through a Pinner-like reaction, reversible thioimidate adducts with biological nucleophiles such as cysteines [61]. Thus, both compounds (**37**) and (**38**) contain warheads able to establish covalent, reversible interactions with nucleophilic cysteine residues. On the other hand, even though irreversible inhibition was achieved by the carbamate derivative (**37**), only a partial irreversible effect was observed with compound (**38**), suggesting that reversibility of inhibition can, and should, be modulated by several chemical factors for the development of these classes of compounds.

Irreversible inhibition of EGFR has also been described for the boron-conjugated 4-anilinoquinazoline (**39**) [62], Fig. (**8**). Compound (**39**) showed a time-dependent inhibition of EGFR autophosphorylation, with an effect that persists 5 h after the removal of the compound from the reaction medium. Mechanistic modeling simulations within the ATP-binding site of the EGFR kinase domain suggested that the boronic portion of compound (**39**) is able to form a covalent B-O bond with Asp800 and hydrogen bonds with Cys797 and Asp800. These interactions may cause the observed prolonged inhibition of EGFR kinase by compound (**39**) [62].

CONCLUSIONS

Irreversible inhibitors of EGFR and other erbB receptors constitute an interesting class of compounds, with a promising potential for the therapy of gefitinib-relapsing tumors and for imaging purposes [63]. The chemical strategy consisting of a link between a driver portion, addressed at the ATP-binding site of the kinase domain, and a warhead, aimed at the formation of a covalent bond with a conserved cysteine residue, has proved efficient both *in vitro* and *in vivo*. However, the clinical usefulness of these compounds has been recently questioned by several observations, ranging from tumor cell lines becoming resistant toward irreversible inhibitors [23, 64] to the emergence of target-related toxicity and pharmacokinetic problems [43]. In this scenario, even if some problems could result difficult to overcome, further investigation on the structure-activity and structure-reactivity relationships may provide useful information for compounds with improved selectivity, pharmacokinetics and/or pharmacodynamic properties. In

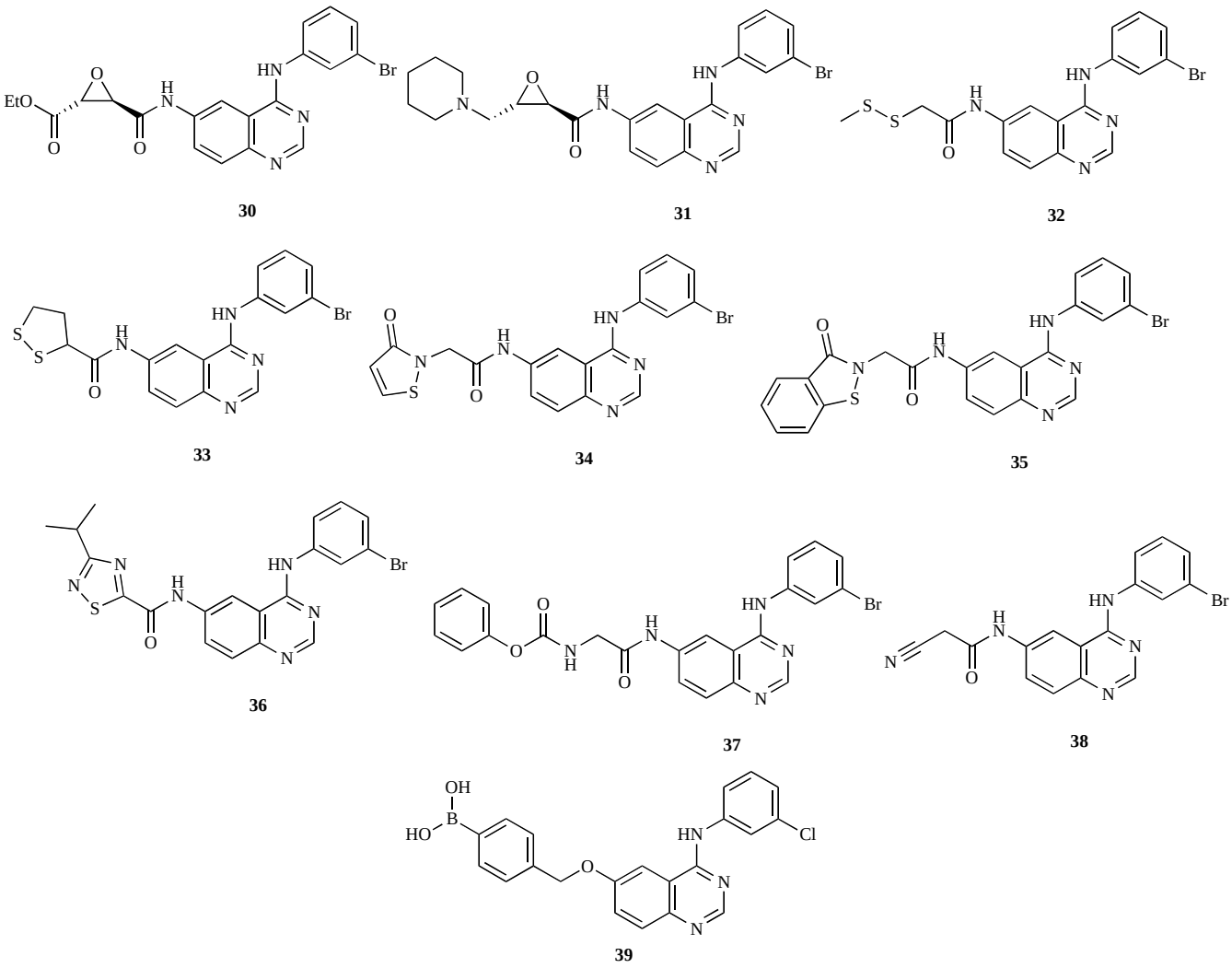


Fig. (8). Other classes of irreversible EGFR/erbB2 inhibitors.

fact, increased reactivity of the Michael acceptor can result in increased background alkylation of cellular thiols, such as glutathione, and therefore a reduction in cellular potency for inhibition of EGFR. Thus, a balance is required to provide compounds that are still capable of rapid alkylation of the target Cys797 without displaying significantly increased background alkylation. In this regard, the chemical space of warheads for EGFR inhibitors is no more confined to acrylamide or butynamide derivatives, and new groups could offer new opportunities to improve the biological properties for this class of compounds, as well as to deepen our knowledge about the mechanism of covalent protein-ligand interactions.

ABBREVIATIONS

EGFR	=	epidermal growth factor receptor
erbB2	=	human epidermal growth factor 2
NSCLC	=	non-small-cell lung cancer
IGF-1R	=	insulin-like growth factor 1 receptor
ATP	=	Adenosine-5'-triphosphate

REFERENCES

[1] Yarden, Y.; Sliwknowski, M.X. Untangling the ErbB signaling network. *Nat. Rev. Mol. Cell. Biol.*, **2001**, *2*, 127-37.

[2] Hynes, N.E.; Lane, H.A. ErbB receptors and cancer: the complexity of targeted inhibitors. *Nat. Rev. Cancer*, **2005**, *5*, 341-54.

[3] Schlessinger, J. Cell signaling by receptor tyrosine kinases. *Cell.*, **2000**, *103*, 211-25.

[4] Olayioye, M.A.; Neve, R.M.; Lane, H.A.; Hynes, N.E. The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J.*, **2000**, *19*, 3159-67.

[5] Mendelson, J.; Balsega, J. Epidermal growth factor receptor targeting in cancer. *Semin. Oncol.*, **2006**, *33*, 369-85.

[6] Salomon, D.S.; Brandt, R.; Ciardello, F.; Normanno, N. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit. Rev. Oncol. Hematol.*, **1995**, *19*, 183-232.

[7] Teman, S.; Kawaguchi, H.; El-Naggar, A.K.; Jelinek, J.; Tang, H.; Liu, D.D.; Lang, W.; Issa, J.P.; Lee, J.J.; Mao, L. Epidermal growth factor receptor copy number alterations correlate with poor clinical outcome in patients with head and neck squamous cancer. *J. Clin. Oncol.*, **2007**, *25*, 2164-70.

[8] Baselga, J.; Arteaga, C.L. Critical update and emerging trends in epidermal growth factor receptor targeting in cancer. *J. Clin. Oncol.*, **2005**, *23*, 2445-59.

[9] Johnson, B.E.; Jänne, P.A. Epidermal growth factor receptor mutations in patients with non-small-cell lung cancer. *Cancer Res.*, **2005**, *65*, 7525-9.

[10] Kobayashi, S.; Boggon, T.J.; Dayaram, T.; Jänne, P.A.; Kocher, O.; Meyerson, M.; Johnson, B.E.; Eck, M.J.; Tenen, D.G.; Halmos, B. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.*, **2005**, *352*, 786-92.

[11] Chan, S.K.; Gullick, W.J.; Hill, M.E. Mutations of the epidermal growth factor receptor in non-small cell lung cancer – search and destroy. *Eur. J. Cancer*, **2006**, *42*, 17-23.

[12] Yun, C.H.; Boggon, T.J.; Li, Y.; Woo, M.S.; Greulich, H.; Meyerson, M.; Eck, M.J. Structures of lung cancer-derived mutants and inhibitor complexes: mechanism of activation and insights into differential inhibitor sensitivity. *Cancer Cell.*, **2007**, *11*, 217-27.

[13] Barker, A.J.; Gibson, K.H.; Grundy, W.; Godfrey, A.A; Barlow, J.J.; Healy, M.P.; Woodburn, J.R.; Ashton, S.E.; Curry, B.J.; Sarlett, L.; Henthorn, L.; Richards, L.; Studies leading to the identification of ZD1839 (Iressa): an orally active, selective epidermal growth factor receptor tyrosine kinase inhibitor targeted to the treatment of cancer. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 1911-4

[14] Moyer, J.D.; Barbacci, E.G.; Iwata, K.K.; Arnold, L.; Boman, B.; Cunningham, A.; Di Orio, C.; Doty, J.; Morin, M. J.; Moyer, M. P.; Neveu, M.; Pollack, V.A.; Pustilnick, L.R.; Reynolds, M.M.; Sloan, D.; Theleman, A.; Miller, P.; Induction of apoptosis and cell cycle arrest by CP-358,774, an inhibitor of epidermal growth factor receptor tyrosine kinase. *Cancer Res.*, **1997**, *57*, 4838-48.

[15] Lynch, T.J.; Bell, D.W.; Sordella, R.; Gurubhagavatula, S.; Okimoto, R.A.; Brannigan, B.W.; Harris, P.L.; Haserlat, S.M.; Supko, J G.; Haluska, F.G.; Louis, D.N.; Christiani, D.C.; Settleman, J.; Haber; D.A. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.*, **2004**, *350*, 2129-39.

[16] Pao, W.; Miller, V.A.; Politi, K.A.; Riely, G.J.; Somwar, R.; Zakowski, M.F.; Heelan, R.T.; Kris, M.G.; Varmus, H.E. *KRAS* mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. *PLoS Med*, **2005**, *2*, e17.

[17] Engelman, J.A.; Jänne, P.A. Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. *Clin. Cancer Res.*, **2008**, *14*, 2895-9.

[18] Yun, C.-H.; Mengwasser, K.E.; Toms, A.V.; Woo, M.S.; Greulich, H.; Wong, K.-K.; Meyerson, M.; Eck, M.J. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. *Proc. Natl. Acad. Sci. U.S.A.*, **2008**, *105*, 2070-5.

[19] Camp, E.R.; Summy. J.; Bauer, T.V.; Liu, W.; Gallick, G.E.; Ellis, L.M. Molecular mechanisms of resistance to therapies targeting the epidermal growth factor receptor. *Clin. Cancer Res.*, **2005**, *11*, 397-405.

[20] Engelman, J.F.; Settleman, J. Acquired resistance to tyrosine kinase inhibitors during cancer therapy. *Curr. Opin. Gen Dev.*, **2008**, *18*, 73-9.

[21] Kwak, E.L.; Sordella, R.; Bell, D.W.; Godin-Heymann, N.; Okimoto, R.A.; Branningan, B.W.; Harria, P.L.; Driscoli, D.R.; Fidias, P.; Lynch, T.J.; Rabindran, S.K.; McGinnis, J.P.; Sharma, S.V.; Isselbacher, K.J.; Settleman, J.; Haber, D.A. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. *Proc. Natl. Acad. Sci. U.S.A.*, **2005**, *102*, 7665-70.

[22] Carter, T.A.; Wodicka, L.M.; Shah, N.P.; Velasco, A.M.; Fabian, M.A.; Treiber, D.K.; Milanov, Z.V.; Atteridge, C.E.; Biggs, V.H.; Edeen, P.T.; Floyd, M.; Ford, J.M.; Grotzfeld, R.M.; Herrgard, S.; Insko, D.E.; Mehta, S.A.; Patel, H.K.; Pao, W.; Sawyers, C.L.; Varmus, H.; Zarrinkar, P.P.; Lockhart, D.J. Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. *Proc. Natl. Acad. Sci. U.S.A.*, **2005**, *102*, 11011-6.

[23] Godin-Heymann, N.; Ulkus, L.; Brannigan, B.W.; McDermott, U.; Lamb, J.; Maheswaran, S.; Settleman, J.; Haber, D.A. The T790M "gatekeeper" mutation in EGFR mediates resistance to low concentration of an irreversible EGFR inhibitor. *Mol. Cancer Ther.*, **2008**, *7*, 874-9.

[24] Fry, D.W.; Bridges, A.J.; Denny, W.A.; Doherty, A.; Greis, K.D.; Hicks, J.L.; Hook, K.E.; Keller, P.R.; Leopold, W.R.; Loo, J.A.; McNamara, D.J.; Nelson, J.M.; Sherwood, V.; Smaill, J.B.; Trumpp-Kallmeyer, S.; Dobrusin, E.M. Specific, irreversible inactivation of the epidermal growth factor receptor and erbB2, by a new class of tyrosine kinase inhibitor. *Proc. Natl. Acad. Sci. U.S.A.*, **1998**, *95*, 12022-7.

[25] Blair, J.A.; Rauh, D.; Kung, C.; Yun, C.-H.; Fan, Q.-W.; Rode, H.; Zhang, C.; Eck, M.J.; Weiss, W.A.; Shokat, K. M. Structure-guided development of affinity probes for tyrosine kinases using chemical genetics. *Nature Chem. Biol.*, **2007**, *3*, 229-38.

[26] Singh, J.; Petter, R.C.; Kluge, A.F. Targeted covalent drugs of the kinase family. *Curr. Opinion Chem. Biol.*, **2010**, *14*, 475-80.

[27] Wood, E.R. Truesdale, A.T.; McDonald, O.B.; Yuan, D.; Hassell, A.; Dickerson, S.H.; Ellis, B.; Pennisi, C.; Horne, E.; Lackey, K.; Alligood, K.J.; Rusnak, K.D.; Gilmer, T.M.; Shewchuk, L. A unique structure for epidermal growth factor receptor bound to GW572016 (lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. *Cancer Res.*, **2004**, *64*, 6652-9.

[28] Govindan, R. A review of epidermal growth factor receptor/HER2 inhibitors in the treatment of patients with non-small-cell lung cancer. *Clin. Lung Cancer*, **2010**, *11*, 8-12.

[29] Slichenmyer, W.J.; Elliott, W.L.; Fry, D.W. CI-1033, a pan-erbB tyrosine kinase inhibitor. *Semin. Oncol.*, **2001**, *28*, 80-5.

[30] Minkovsky, N.; Berezov, A. BIBW-2992, a dual receptor tyrosine kinase inhibitor for the treatment of solid tumours. *Curr. Opin. Invest. Drugs*, **2008**, *9*, 1336-46.

[31] Suzuki, T.; Fujii, A.; Ohya, J.; Amano, Y.; Kitano, Y.; Abe, D.; Nakamura, H. Pharmacological characterization of MP-412 (AV-412), a dual epidermal growth factor receptor and erbB2 tyrosine kinase inhibitor. *Cancer Sci.*, **2007**, *98*, 1977-84.

[32] Rabindran, S.K.; Discafani, C.M.; Rosfjord, E.C.; Baxter, M.; Floyd, M.B.; Golas, J.; Hallett, W.A.; Johnson, B.D.; Nilakantan, D.; OverBeek, E.; Reich, M.F.; Shen, R.; Shi, X.; Tsou, H.R.; Wang, Y.F.; Wissner, A. Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the Her-2 tyrosine kinase. *Cancer Res.*, **2004**, *64*, 3958-65.

[33] Erlichman, C.; Hidalgo, M.; Boni, J.P.; Martins, P.; Quinn, S.E.; Zacharchuk, C.; Amorusi, P.; Adjei, A.A.; Rowinsky, E.K. Phase I study of EKB-569, an irreversible inhibitor of the epidermal growth factor receptor, in patients with advanced solid tumors. *J. Clin. Oncol.*, **2006**, *24*, 2252-60.

[34] Stamos, J.; Sliwkowski, M.X.; Eigenbrot, C. Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor. *J. Biol. Chem.*, **2002**, *277*, 46265-72.

[35] Smaill, J.B.; Showalter, H.D.H.; Zhou, H.; Bridges, A.J.; McNamara, D.J.; Fry, D.W.; Nelson, J.M.; Sherwood, V.; Vincent, P.W.; Roberts, B.J.; Elliot, W.L.; Denny, W.A. Tyrosine kinase inhibitors. 18. 6-Substituted 4-anilinoqionazolines and 4-anilinopyrido[3,4-*d*]pyrimidines as soluble, irreversibile inhibitors of the epidermal growth factor receptor. *J. Med. Chem.*, **2001**, *44*, 429-40.

[36] Tsou, H.-R.; Mamuyra, N.; Johnson, B.D.; Reich M.F.; Gruber, B.C.; Ye, F.; Nilakantan, R.; Shen, R.; Discafani, C.; DeBlanc, R.; Davis, R.; Koehn, F.E.; Greenberger, L.M.; Wang, Y.-F.; Wissner, A. 6-substituted-4-(3-bromophenylamino)quinazolines as putative irreversible inhibitors of the epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor (HER-2) tyrosine kinase with enhanced antitumor activity. *J. Med. Chem.*, **2001**, *44*, 2719-34.

[37] Smaill, J.B.; Rewcastle, G.W; Loo, J.A.; Greis, K.D.; Chan, O.H.; Reyner, E.L.; Lipka, E.; Showalter, H.D.H.; Vincent, P.W.; Elliot, W.L.; Denny, W. Tyrosine kinase inhibitors. 17. Irreversible inhibitors of the epidermal growth factor receptor: 4-(phenylamino)quinazoline- and 4-(phenylamino)pyrido[3,2-*d*]pyrimidine-6-acrylamides bearing additional solubilizing functions. *J. Med. Chem.*, **2000**, *43*, 1380-97.

[38] Cha, M.Y.; Lee, K.-O.; Kim, J.W.; Lee, C.G.; Song, J.Y.; Kim, Y.H.; Lee, G.S.; Park, S.B.; Kim, M.S. Discovery of a novel Her-1/Her-2 dual tyrosine kinase inhibitor for the treatment of Her-1 selective inhibitor-resistant non-small cell lung cancer. *J. Med. Chem.*, **2009**, *52*, 6880-8.

[39] Wissner, A.; Tsou, H.-R.; Jhonson, B.D.; Hamann, P.R.; Zhang, N. Substituted quinazoline derivatives and their use as tyrosine kinase inhibitors. World Pat. Appl. 9909016, February 1999.

[40] 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.; Rabindran, S.K.; Shen, R.; Shi, X.; Wang, Y.-F.; Upeslacis, J.; Wissner, A. 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*, 1107-31.

[41] Wissner, A.; Overbeek, E.; Reich, M.F.; Floyd, B.; Jhonson, B.D.; Mamuya, N.; Rosfjord, E.C.; Discafani, C.; Davis, R.; Shi, X.; Rabindran, S.K; Gruber, F.Y.; Hallet, W.A.; Nilakantan, R.; Shen, R.; Wang, Y.-F.; Greenberger, L.M.; Tsou, H.-R. Synthesis and structure-activity relationships of 6,7-disubstituted 4-anilinoquinoline-3-carbonitriles. The design o fan orally active, irreversible inhibitor of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR) and the human epidermal growth factor-2 (HER-2). *J. Med. Chem.*, **2003**, *46*, 49-63.

[42] Yap, T.A.; Vidal, L.; Adam, J.; Stephens, P.; Spicer, J.; Shaw, H.; Ang, J.; Temple, G.; Bell, S.; Shahidi, M.; Uttenreuther-Fischer, M.; Stopfer, P.; Futreal, A.; Calvert, H.; de Bono, J.S.; Plummer, R. Phase I Trial of the irreversible EGFR and HER2 kinase inhibitor BIBW 2992 in patients with advanced solid tumors. *J. Clin. Oncol.*, **2010**, *28*, 3965-72.

[43] Sequist, L.V.; Besse, B.; Lynch, T.J.; Miller, V.A.; Wong, K.K.; Gitlitz, B.; Eaton, K.; Zacharchuk, C.; Freyman, A.; Powell, C.; Ananthakrishnan, R.; Quinn, S.; Soria, J.C. Neratinib, an irreversible pan-ErbB receptor tyrosine kinase inhibitor: results of a phase II trial in patients with advanced non-small-cell lung cancer. *J. Clin. Oncol.*, **2010**, *28*, 3076-83.

[44] Ranson, M.; Hammond, L.A.; Ferry, D.; Kris, M.; Tullo, A.; Murray, P.I.; Miller, V.; Averbuch, S.; Ochs, J.; Morris, C.; Feyereislova, A.; Swaisland, H.; Rowinsky, E.K. ZD1839, a selective oral epidermal growth factor receptor-tyrosine kinase inhibitor, is well tolerated and active in patients with solid, malignant tumors: results of a phase I trial. *J. Clin. Oncol.*, **2002**, *20*, 2240-50.

[45] Hidalgo, M.; Siu, L.L.; Nemunaitis, J.; Rizzo, J.; Hammond, L.A.; Takimoto, C.; Eckhardt, S.G.; Tolcher, A.; Britten, C.D.; Denis, L.; Ferrante, K.; Von Hoff, D.D.; Silberman, S.; Rowinsky, E.K. Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. *J. Clin. Oncol.*, **2001**, *19*, 3267-79.

[46] Burris, H.; Hurwitz, H.; Dees, E.; Dowlati, A.; Blackwell, K.; O'Neil, B.; Marcom, P.; Ellis, M.; Overmoyer, B.; Jones, S.; Harris, J.; Smith, D.; Koch, K.; Stead, A.; Mangum, S.; Spector, N. 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*, 5305-13.

[47] Smaill, J.B.; Palmer, B.D.; Rewcastle, G.W.; Denny, W.A.; McNamara, D.J.; Dobrusin, E.M.; Bridges, A.J.; Zhou, H.; Showalter, H.D.H.; Winters, R.T.; Leopold, W.R.; Fry, D.W.; Nelson, J.M.; Slintak, V.; Elliot, W.L.; Roberts, B.J.; Vincent, P.W.; Patmore, S.J. Tyrosine kinase inhibitors. 15. 4-(phenylamino)quinazoline and 4-(phenylamino)pyrido[*d*]pyrimidine acrylamides as irreversible inhibitors of the ATP binding site of the epidermal growth factor receptor. *J. Med. Chem.*, **1999**, *42*, 1803-15.

[48] Wissner, A.; Hamann, P.R.; Nilakantan, R.; Greenberger, L.M.; Ye, F.; Rapuano, T.A.; Loganzo, F. Synthesis and EGFR kinase inhibitory activity of 6-substituted-4-anilino[1,7] and [1,8]naphthyridine-3-carbonitriles. *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 1411-6.

[49] Zhou, W.; Ercan, D.; Chen,L.; Yun, C.-H.; Li, D.; Capelletti, M; Cortot, A.B.; Chierac, L.; Iacob, R.E.; Padera, R.; Engen, J.R.; Wong, K.-K.; Eck, M.J.; Gray, N.S.; Jänne, P. Novel mutant-selective EGFR kinase inhibitors against EGFR T790M. *Nature*, **2009**, *462*, 1070-4.

[50] Discafani, C.M.; Carroll, M.L.; Floyd Jr., M.B.; Hollander, I.J.; Husain, Z.; Johnson, B.D.; Kitchen, D.; May, M.K.; Malo, M.S.; Minnick Jr., A.A.; Nilakantan, R.; Shen, R.; Wang, Y.-F.; Wissner, A.; Greenberger, L.M. Irreversible inhibition of epidermal growth factor receptor tyrosine kinase with *in vivo* activity by *N*-[4-[(3-bromophenyl)amino]-6-quinazolinyl]-2-butynamide (CL-387,785). *Biochem. Pharmacol.*, **1999**, *57*, 917-25.

[51] Klutchko, S.R.; Zhou, H.; Winters, R.T.; Tran, T.P.; Bridges, A.J.; Althaus, I.W.; Amato, D.M.; Elliott, W.L.; Ellis, P.A.; Meade, M.A.; Roberts, B.J.; Fry, D.W.; Gonzales, A.J.; Harvey, P.J.; Nelson, J.M.; Sherwood, V.; Han, H.-K.; Pace, G.; Smaill, J.B.; Denny, W.A.; Showalter, H.D.H. Tyrosine kinase inhibitors. 19. 6-alkynamides of 4-anilinoquinazolines and 4-anilinopyrido[3,4-*d*]pyrimidines as irreversible inhibitors of the erbB family of tyrosine kinase receptors. *J. Med. Chem.*, **2006**, *49*, 1475-85.

[52] Mishani, E.; Abourbeh, G.; Jacobson, O.; Dissoki, S.; Ben Daniel, R.; Rozen, Y.; Shaul, M.; Levitzki, A. High-affinity epidermal growth factor receptor (EGFR) irreversible inhibitors with diminished chemical reactivities as positron emission tomography (PET)-imaging agent candidates of EGFR overexpressing tumors. *J. Med. Chem.*, **2005**, *48*, 5337-48.

[53] Carmi, C.; Cavazzoni, A.; Vezzosi, S.; Bordi, F.; Vacondio, F.; Silva, C.; Rivara, S.; Lodola, A.; Alfieri, R.R.; La Monica, S.; Galetti, M.; Ardizzoni, A.; Petronini, P.G.; Mor, M. Novel irreversible epidermal growth factor receptor inhibitors by chemical modulation of the cysteine-trap portion. *J. Med. Chem.*, **2010**, *53*, 2038-50.

[54] Matheson, S.L.; McNamee, J.; Jean-Claude, B.J. Design of a chimeric 3-methyl-1,2,3-triazene with mixed receptor tyrosine kinase and DNA damaging properties: a novel tumour targeting strategy. *J. Pharmacol. Exp. Ther.*, **2001**, *296*, 832-40.

[55] Cavazzoni, A.; Alfieri, R.; Carmi, C.; Zuliani, V.; Galetti, M.; Fumarola, C.; Frazzi, R.; Bonelli, M.; Bordi, F.; Lodola, A.; Mor, M.; Petronini, P.G. Dual mechanism of action of the 5-benzylidene-hydantoin UPR1204 on lung cancer cell lines. *Mol. Cancer Ther.*, **2008**, *7*, 361-70.

[56] Wood, E.R.; Shewchuk, L.M.; Ellis, B.; Brignola, P.; Brashear, R.L.; Caferro, T.R.; Dickerson, S.H.; Dickson, H.D.; Donaldson, K.H.; Gaul, M.; Griffin, R.J.; Hassell, A.M.; Keith, B.; Mullin, R.; Petrov, K.G.; Reno, M.J.; Rusnak, D.W.; Tadepalli, S.M.; Ulrich, G.C.; Wagner, C.D.; Vanderwall, D.E.; Waterson, A.G.; Williams, J.D.; White, W.L.; Uehling, D.E. 6-Ethynylthieno[3,2-*d*]- and 6-ethynylthieno[2,3-*d*]pyrimidin-4-anilines as tunable covalent modifiers of ErbB kinases. *Proc. Natl. Acad. Sci. U.S.A.*, **2008**, *105*, 2773-8.

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

[58] 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.; Dickerson, S.H.; Rusnak, D.W.; Spehar, G.M.; Wood, E.R., Griffin, R.J.; Uehling, D.E. Synthesis and evaluation of aniline headgroups for alkynyl thienopyrimidine dual EGFR/ErbB-2 kinase inhibitors. *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 1332-6.

[59] Antonello, A.; Tarozzi, A.; Morroni, F.; Cavalli, A.; Rosini, M.; Hrelia, P.; Bolognesi, M. L.; Melchiorre, C. Multitarget-directed drug design strategy: a novel molecule designed to block Epidermal Growth Factor Receptor (EGFR) and to exert proapoptotic effects. *J. Med. Chem.*, **2006**, *49*, 6642-5.

[60] Potashman, M.H.; Duggan, M.E. Covalent Modifiers: an orthogonal approach to drug design. *J. Med. Chem.*, **2009**, *52*, 1231-46.

[61] Powers, J.C.; Asgian, J.L.; Dogan Ekici, O.; Ellis James, K. Irreversible inhibitors of serine, cysteine, and threonine proteases. *Chem. Rev.*, **2002**, *102*, 4639-750.

[62] Ban, H.S.; Usui, T.; Nabeyama, W.; Morita, H.; Fukuzawa, K.; Nakamura, H. Discovery of boron-conjugated 4-anilinoquinazoline as a prolonged inhibitor of EGFR tyrosine kinase. *Org. Biomol. Chem.*, **2009**, *7*, 4415-27.

[63] Gelovani, J.G. Molecular imaging of epidermal growth factor receptor expression-activity at the kinase level in tumors with positron emission tomography. *Cancer Metastasis Rev.*, **2008**, *27*, 645-53.

[64] Ercan, D.; Zejnullahu, K.; Yonesaka, K.; Xiao, Y.; Cappelletti, M.; Rogers, A.; Lifshits, E.; Brown, A.; Lee, C.; Christensen, J.G.; Kwiatkowski, D.J.; Engelman, J.A.; Jänne, P.A. Amplification of EGFR T790M causes resistance to an irreversible EGFR inhibitor. *Oncogene*, **2010**, *29*, 1-11.

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