Allosteric Inhibitor TREA-0236 Containing Non-hydrolysable Quinazoline-4-one for EGFR T790M/C797S Mutants Inhibition

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Non-small cell lung cancer (NSCLC) is one of the leading causes of cancer-related death globally.[1,2] Epidermal growth factor receptor (EGFR) activating mutations such as EGFR L858R and EGFR ex19 deletions are responsible for 30–40% of NSCLC patients in East Asian populations and 10–15% in European descendants.[3] All the approved EGFR-tyrosine kinase inhibitors (TKIs) give clinical benefits to EGFR-related NSCLC patients in terms of overall survival and quality of patient life (Figure 1).[4,5] However so far approved EGFR-TKIs are ATP catalytic site inhibitors and their benefit is ultimately limited due to mutational resistance near the catalytic site.[6–8] In a recent osimertinib and olmutinib clinical study, newly acquired resistance "EGFR C797S mutation" has been reported.[9,10] Since second and third generation EGFR-TKIs are featured as irreversible inhibitors to the EGFR C797, there are no more effective treatment options to the EGFR C797S mutation. Therefore there is an urgent unmet medical need for next generation EGFR-TKIs via non-irreversible inhibition to EGFR T790M/C797S mutations.[11]

An alternative strategy can be inhibition in sites other than the ATP catalytic site.[12] Novel EGFR allosteric inhibitors, EAI001 and EAI045, have been recently reported (Figure 2).[13] They bind to an allosteric pocket created in the C-helix inactive conformation of the EGFR mutants, and are biochemically potent to both EGFR L858R/T790M and EGFR L858R/T790M/C797S mutants while sparing wild-type EGFR activity. Their cellular potency is synergistically boosted when co-treated with EGFR antibody which blocks EGFR asymmetric dimeric formation. Since EAI045 shows strong inhibition to EGFR L858R/T790M and EGFR L858R/T790M/C797S mutants, developing EGFR allosteric inhibitors provides new hope to treat EGFR C797S-mutated NSCLC patients.

EAI045 has a di-peptide core with *N*-terminal aminothiazole. Peptides are metabolically inherited unstable and the resulting 2-aminothiazole metabolites are *BAD*: 2-aminothiazoles are known as promiscuous hitting scaffold in high throughput screening (HTS) and cause methemoglobinemia toxicity and reactive intermediate formation leading to substantial covalent protein binding.[14,15]

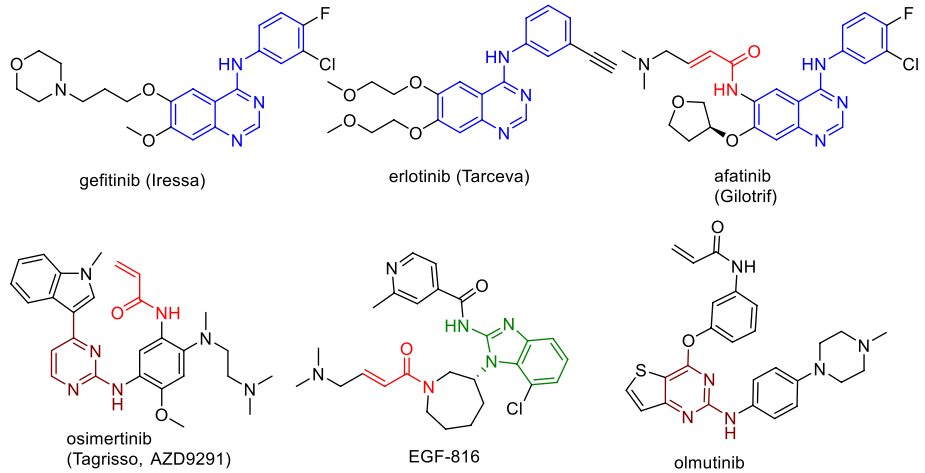


Figure 1. Various EGFR inhibitors.

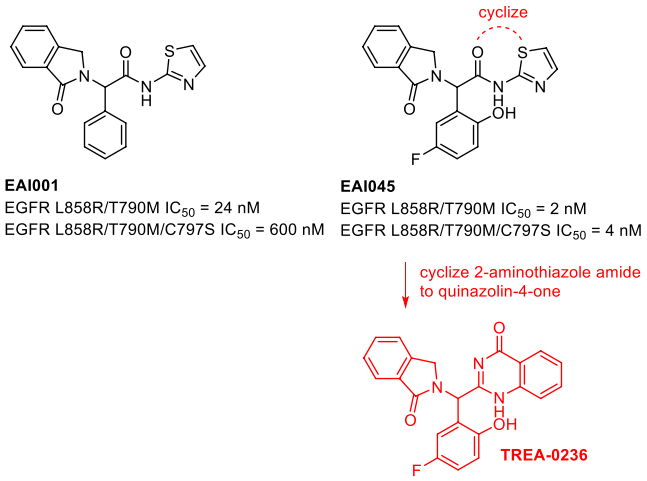


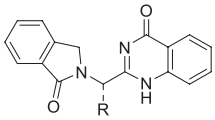
Figure 2. EGFR allosteric inhibitors and non-peptidic scaffold hopping (drawn in red).

Therefore non-peptidic replacement of 2-aminothiazole amide bond would be ideal for its improved safety and pharmacokinetic & pharmacodynamic properties.

Based on EAI045 structure, we designed **TREA-0236** featured with a quinazoline-4-one instead of the 2-aminothiazole amide, so that the unwanted metabolite formation is structurally prohibited.[13] In order to investigate the binding mode of the quinazoline-4-one inhibitors, a molecular docking study was first examined (Figure 3). Molecular modeling studies were performed using the Glide software in the Maestro11.5 software with standard precision (SP) options.[16] The crystal structure of EGFR T790M/V948R kinase retrieved from Protein Data Bank (PDB code: 5D41) was used as the receptor model. The binding pose of co-crystal ligand was regenerated with a good root mean square displacement of 0.1 Å, showing the robustness of our docking protocols. The predicted binding pose of **TREA-0236** is nearly identical to the crystal structure of EAI001 which is located next to ATP binding site: **TREA-0236** retains the key hydrogen bonding between the quinazoline-4-one N1 and the carboxylate of Asp855. In addition, hydrophobic interactions are also important for ligand binding. The heterocyclic quinazoline-4-one is positioned in the hydrophobic pocket formed by Met790, Ala743, Val726, and Lys745. The isoindolin-1-one ring is stabilized by Leu788, Met766, and Leu858. The phenyl group enjoys hydrophobic interaction with Met766 and Leu777, and its phenolic OH makes additional hydrogen bond to Phe856 amide carbonyl.

Encouraged by computational modeling, we performed a medicinal chemistry campaign. Synthetic procedure of representative molecule **TREA-0236** is outlined in Scheme 1. 2-Amino-2-(5-fluoro-2-methoxyphenyl)acetic acid **2** was prepared from 5-fluoro-2-methoxybenzaldehyde. 5-Fluoro-2-methoxybenzaldehyde was condensed with potassium cyanide and ammonium carbonate to provide 5-(5-fluoro-2-methoxyphenyl)imidazolidine-2,4-dione **1** in high yield. Saponification of **1** with potassium hydroxide under reflux yielded 2-amino-2-(5-fluoro-2-methoxyphenyl)acetic acid **2** as a powder.[17] Condensation of **2** with phthalaldehyde in acetic acid afforded 2-(5-fluoro-2-methoxyphenyl)-2-(1-oxoisoindolin-2-yl)acetic acid **3** as gray solid. The acid **3** was then coupled with 2-aminobenzamide using HATU

Table 1. Biochemical activities of substituted quinazoline-4-one derivatives to EGFR L858R/T790M/C797S.



Compound	R	EGFR L858R/T790 M/C797S % inh. @10 μM	EGFR L858R/T790 M/C797S IC_{50} (μM)
EAI001	—		0.6
EAI045	—	99	0.004
6	Ph	1	>10
7	3-F-Ph	3	>10
8	2-OH-Ph	37	>10
TREA-0236	5-F-2-OH-Ph	80	5.3
9	5-Cl-2-OH-Ph	32	>10
10	H	7	>10
11	(S)-Me	0	>10
12	(S)-*i*-Pr	5	>10
13	(S)-*i*-Bu	4	>10
14	(R)-*i*-Bu	0	>10
15	(S)-$CH_2CH_2SCH_3$	0	>10
16	(R)-$CH_2CH_2SCH_3$	0	>10
17	(S)-CH_2Ph-*p*-OH	0	>10
18	(S)-$CH_2CH_2CONH_2$	18	>10

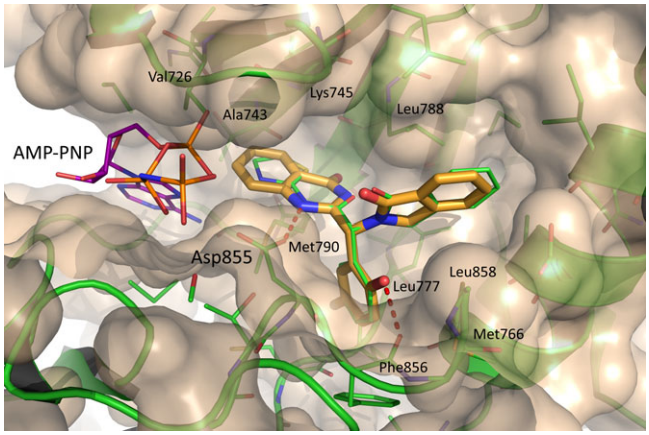
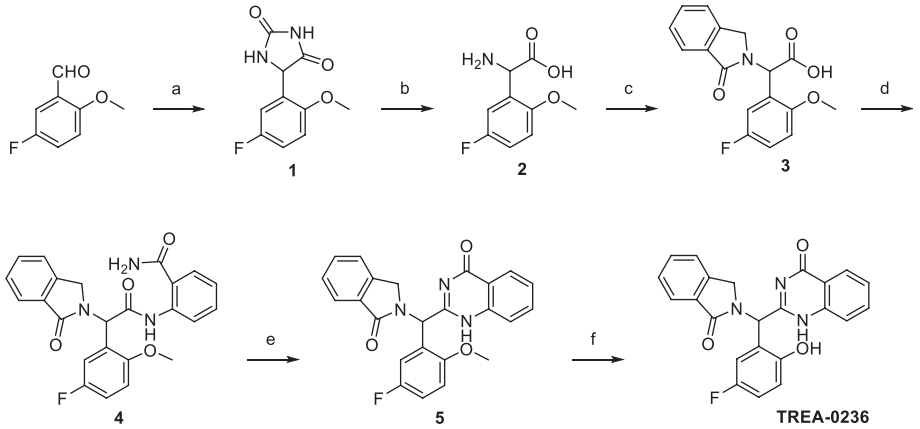


Figure 3. Proposed binding mode of **TREA-0236** (thick yellow sticks) and EAI001 (thin green lines) in the EGFR T790M/V948R. EGFR is drawn by ribbons along with the interacting residues represented as sticks, and hydrogen-bonding interactions are marked with dashed red lines.



Scheme 1. Reagents and conditions: (a) KCN, $(NH_4)_2CO_3$, H_2O/EtOH = 1/2.5, 60°C, 5 h, (92%); (b) KOH, 130°C, 60 h, (55%); (c) phthalaldehyde, AcOH, 110°C, 10 min, (54%); (d) 2-aminobenzamide, HATU, DIPEA, DMF, rt, 6 h; (e) NaOH, THF, reflux, overnight, (39%, 2 steps); (f) BBr_3, CH_2Cl_2, −78°C to rt, 20 min, (47%).

in *N,N*-dimethylformamide to give crude 2-(2-(5-fluoro-2-methoxyphenyl)-2-(1-oxoisoindolin-2-yl)acetamido)benzamide **4**. Quinazolin-4(1*H*)-one cyclization of **4** in the presence of sodium hydroxide provided 2-((5-fluoro-2-methoxyphenyl)(1-oxoisoindolin-2-yl)methyl)quinazolin-4(1*H*)-one **5** (2 step yields: 39%).[18] Finally, anisole **5** was de-methylated using boron tribromide to make the desired 2-((5-fluoro-2-hydroxyphenyl)(1-oxoisoindolin-2-yl) methyl)quinazolin-4(1*H*)-one **TREA-0236** in 47% yield. All the other analogs, **6**–**18**, have been prepared through this chemistry route (see Supporting Information).

The synthesized compounds were evaluated with EGFR L858R/T790M/C797S biochemical inhibition assay.[19] Disappointingly, all of the molecules showed more than 10 μM inhibition concentration except **TREA-0236** (IC_{50} = 5.3 μM) which is a close analog to EAI045. Compound **6**, the close analog of EAI001, was inactive in our assay. *Meta*-fluorophenyl analog **7** and *ortho*-hydroxylphenyl analog **8** were not active either at 10 μM. Chlorine displacement analog **9** instead of fluorine on **TREA-0236** did not improve biochemical activity. In addition to substituted phenyl rings, proton, and alkyl substitutions analogs (**10**–**18**), derived from natural aminoacids, were prepared and tested. However, all the proton and alkyl substitutions resulted in near complete loss of biochemical activity. Based on current research results, the 5-fluoro-2-hydroxylphenyl substitution on the quinazoline-4-one scaffold is essential for the EGFR L858R/T790M/C797S mutant activity. This observation may be attributed to additional hydrogen bond interaction between the phenolic OH and Phe856 amide carbonyl as depicted in Figure 3.

So far little is known about the EAI045 structure–activity-relationship (SAR). We have attempted to replace the undesirable aminothiazole with non-hydrolysable quinazoline-4-one on the EAI045 for improved drug properties, however only **TREA-0236** shows a level of biochemical activity and it is inferior to parent EAI045. Further the non-hydrolysable scaffold search devoid of aminothiazole is currently underway and will be reported in due course.

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Supporting Information. Additional supporting information is available in the online version of this article.

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19. EGFR kinase assay was performed using the europium labeled antibody that specifically recognizes phosphotyrosine as donor dye and streptavidin protein labeled with a small molecular weight fluorophore acceptor dye XL665 provided by Cisbio, Inc. Recombinant EGFR L858R/T790M/C797S mutant was purchased from SignalChem, Inc. All reaction components except ATP were added to the wells first and the reaction was started by adding ATP to the assay mixture containing the enzyme and peptide substrate. After 1 h incubation, the FRET signal between europium cryptate and XL665 was measured to quantify the phosphorylation of the substrate peptide. Envision multilabel reader (Perkin Elmer) was used to measure the fluorescence of the samples at 620 nm (europium-labeled antibody) and 665 nm (XL665 labeled streptavidin) after excitation at 320 nm.

