Supplementary Information

Discovery of a series of 1,2,3-triazole-containing Erlotinib derivatives with potent anti-tumor activities against non-small cell lung cancer (NSCLC)

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Figure S1-2. $^{13}$C NMR spectrum (150 MHz, DMSO-$d_6$) of compound e1
Figure S1-3. HR MS of compound e1

![HR MS of compound e1](image-url)

- RT: 0.14
- AV: 1
- NL: 1.90E6
- F: FTMS + p ESI Full ms [100.0000-1000.0000]
Figure S2-1. $^1$H NMR spectrum (600 MHz, DMSO-$d_6$) of compound e2
Figure S2-2. $^{13}$C NMR spectrum (150 MHz, DMSO-$d_6$) of compound e2
Figure S2-3. HR MS of compound e2
Figure S3-1. $^1$H NMR spectrum (600 MHz, DMSO-d$_6$) of compound e3
Figure S3-2. $^{13}$C NMR spectrum (150 MHz, DMSO-d$_6$) of compound e3
Figure S3.3. HR MS of compound e3

- Retention Time (RT): 0.09
- Average (AV): 1
- Noise Level (NL): 1.40E6

FTMS + p ESI Full ms [100.0000-1000.0000]

Relative Abundance

m/z values and corresponding abundances are shown on the graph.
Figure S4-1. $^1$H NMR spectrum (600 MHz, DMSO-$d_6$) of compound e4
Figure S4-2. $^{13}\text{C}$ NMR spectrum (150 MHz, DMSO-$d_6$) of compound e4
Figure S4-3. HR MS of compound e4

m/z: 682.0, 683.0624, 684.0656, 685.5603, 686.0632

C<sub>29</sub>H<sub>29</sub>O<sub>4</sub>N<sub>6</sub>Br<sub>2</sub> = 683.0612 17.5 RDBE 1.8796 ppm
Figure S5-1. $^1$H NMR spectrum (600 MHz, DMSO-d$_6$) of compound e5
Figure S5-2. $^{13}$C NMR spectrum (150 MHz, DMSO-$d_6$) of compound e5
Figure S5-3. HR MS of compound e5

- **e5-1000**
- **RT:** 0.16-0.18
- **AV:** 3
- **NL:** 2.01E6

**T:** FTMS + c ESI cv=0.00  Full ms [200.0000-1000.0000]
Figure S6-1. $^1$H NMR spectrum (600 MHz, DMSO-$_d_6$) of compound e6
Figure S6.2. $^{13}$C NMR spectrum (150 MHz, DMSO-d$_6$) of compound e6
Figure S6-3. HR MS of compound e6

- **RT:** 0.12
- **AV:** 1
- **NL:** 2
- **F:** FTMS + ESI/Fully (100.0000-1000.0000)

**Molecular Formula:** C$_{28}$H$_{27}$O$_{4}$N$_{6}$F Na

**Exact Mass:** 553.1970

**Exact Mass Error:** 1.6889 ppm

**Relative Abundance:**
- m/z 553.1979
- m/z 554.2007
- m/z 555.2038
Figure S7-1. $^1$H NMR spectrum (600 MHz, DMSO-$d_6$) of compound e7.
Figure S7-2. $^{13}$C NMR spectrum (150 MHz, DMSO-d$_6$) of compound e7
Figure S7-3. HR MS of compound e7
Figure S8-1. $^1$H NMR spectrum (600 MHz, DMSO-$d_6$) of compound e8
Figure S8-2. $^{13}$C NMR spectrum (150 MHz, DMSO-d$_6$) of compound e8
Figure S8-3. HR MS of compound e8
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Figure S9-2. $^{13}$C NMR spectrum (150 MHz, DMSO-$d_6$) of compound e9
Figure S9-3. HR MS of compound e9
Figure S10-1. $^1$H NMR spectrum (600 MHz, DMSO-d$_6$) of compound e10
Figure S10-2. $^{13}$C NMR spectrum (150 MHz, DMSO-$d_6$) of compound e10
Figure S10-3. HR MS of compound e10

Figure showing the mass spectrum of compound e10 with m/z values and corresponding relative abundances. The spectrum displays peaks at m/z 612.1177, 614.1207, 615.1158, and 616.1187.
Figure S11-1. $^1$H NMR spectrum (600 MHz, DMSO-d$_6$) of compound e11
Figure S11-2. $^{13}$C NMR spectrum (150 MHz, DMSO-$d_6$) of compound e11
Figure S11-3. HR MS of compound e11

$\text{C}_{29}\text{H}_{30}\text{O}_{5}\text{N}_{6}\text{Na} = 565.2170$

$17.5$ ppm

$1.105$ ppm
Figure S12-1. $^1$H NMR spectrum (600 MHz, DMSO-d$_6$) of compound e12
Figure S12-2. $^{13}$C NMR spectrum (150 MHz, DMSO-$d_6$) of compound e12
Figure S12-3. HR MS of compound e12

527.2410  C$_{29}$H$_{31}$O$_4$N$_6$ = 527.2401
17.5 RDBE
1.5873 ppm

528.2441
529.2473
530.2484
529.8494
Figure S13-1. $^1$H NMR spectrum (600 MHz, DMSO-d$_6$) of compound a13
Figure S13-2. $^{13}$C NMR spectrum (150 MHz, DMSO-$d_6$) of compound e13
Figure S13-3. HR MS of compound e13

RT: 0.12-0.14  AV: 2  NL: 1.15E6

579.0  579.5  580.0  580.5  581.0  581.5  582.0  582.5  583.0  583.5  584.0
m/z

580.1923 C$_{28}$H$_{27}$O$_{6}$N$_{7}$Na = 580.1915  18.5 RDBE  1.4481 ppm

581.1953

582.1979
Figure S14-1. $^1$H NMR spectrum (600 MHz, DMSO-$d_6$) of compound e14
Figure S14-2. $^{13}$C NMR spectrum (150 MHz, DMSO-$d_6$) of compound e14
Figure S14-3. HR MS of compound e14
Figure S15-1. $^1$H NMR spectrum (600 MHz, DMSO-$d_6$) of compound e15
Figure S15-2. $^{13}$C NMR spectrum (150 MHz, DMSO-d$_6$) of compound e15
Figure S15-3. HR MS of compound e15

- **RT:** 0.14-0.17
- **AV:** 2
- **NL:** 1.47E6
- **F:** FTMS + ESI/Full MS [100.0000-1000.0000]

**Molecular Mass Calculations:**

- **535.2069**
- **C28H28O4N6Na**
- **17.5 RDBE**
- **0.8997 ppm**

- **536.2100**
- **537.2130**
Figure S16-1. $^1$H NMR spectrum (400 MHz, DMSO-d$_6$) of compound e16
Figure S16-2. $^{13}$C NMR spectrum (100 MHz, DMSO-d$_6$) of compound e16
Figure S16-3. HR MS of compound e16

Relative Abundance

603.1945 C_{29}H_{27}O_{4}N_{6}F_{3}Na = 603.1938 17.5 RDBE 1.0758 ppm

604.1974

605.2005
Figure S17-1. $^1$H NMR spectrum (400 MHz, DMSO-d$_6$) of compound e17
Figure S17-2. $^{13}$C NMR spectrum (100 MHz, DMSO-\(d_6\)) of compound e17
Figure S17-3. HR MS of compound e17

C_{30} H_{32} O_6 N_6 Na = 595.2276
17.5 RDBE
1.6158 ppm
Figure S18-1. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$) of compound e18
Figure S18-2. $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$) of compound e18
Figure S18-3. HR MS of compound e18

- RT: 0.12
- AV: 1
- NL: 1.93E6
- FTMS + ESI Full ms [100.0000-1000.0000]
Figure S19-1. $^1$H NMR spectrum (400 MHz, DMSO-d$_6$) of compound e19
Figure S19-2. $^{13}$C NMR spectrum (100 MHz, DMSO-d$_6$) of compound e19
Figure S19-3. HR MS of compound e19

C_{30}H_{32}O_{6}N_{6}Na = 595.2276
17.5 RDBE
0.8980 ppm

594.5
595.0
595.5
596.0
596.5
597.0
597.5
598.0
598.5
599.0

m/z

0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100

Relative Abundance
Figure S20-1. $^1$H NMR spectrum (600 MHz, DMSO-$d_6$) of compound e20
Figure S20-2. $^{13}$C NMR spectrum (150 MHz, DMSO-$d_6$) of compound e20
Figure S20-3. HR MS of compound e20

- RT: 0.17-0.20
- AV: 2
- NL: 1.15E6
- FTMS + p ESI Full ms [100.0000-1000.0000]

Relative Abundance

- 563.2381 C_{30}H_{32}O_{4}N_{6}Na = 563.2377
- 564.2413
- 565.2443
We conducted molecular docking studies to explore the potential binding modes of compound e4 in the active site of EGFR. The docking studies states that the binding energy of erlotinib is $-7.4 \text{kcal/mol}$, and Elotinib formed hydrogen bonds with the target amino acid residues including LYS721, MET769, CYS773 and ASP831, and form hydrophobic interactions with THR766. Results show that e4 binds to EGFR in the ATP binding site mainly composed of hydrophobic residues with docking score of $-9.6 \text{kcal/mol}$. We found that the 3,5-dibromobenzy group introduced to the N3 position of 1,2,3-triazole could occupy the hydrophobic pocket containing LEU 694 and THR 830, and the backbone amino group of MET 769 formed a hydrogen bond with one amino of the 1,2,3-triazole group. The amino group of CYS 773 formed hydrogen
bonds with the quinazoline.

The docking results show that e4 has a desirable binding affinity with EGFR but a different binding mode from that of Erlotinib. However, our results show that the EGFR inhibitory activities of Erlotinib is better, but e4 demonstrates preferable anticancer activities in vitro. Thus, more mechanisms about the anticancer activities of these new compounds should be further studied.

**Methods**

In silico docking was carried out using AutoDock 4.2. Crystal structure of human epidermal growth factor receptor (EGFR) (PDB ID: 1M17) were downloaded from the protein data bank (https://www.rcsb.org/). Pymol (The PyMOL Molecular Graphics System) programs was used to remove all waters, ligands and co-factors. AutoDock Tools was used to add hydrogens, calculate Gasteiger charges, and generate PDBQT files of compounds and receptor. The grid of 54, 54, and 54 points in x, y, and z directions of EGFR were built with a grid spacing of 0.375 Å and a distance-dependent function of the dielectric constant were used for the calculation of the energetic map. The default settings were used for all other parameters. Lamarckian genetic algorithm method was employed for docking simulations. The standard docking procedure was used for a rigid protein and a flexible ligand whose torsion angles were identified (for 200 independent runs per ligand).

**Reference**

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