Design and discovery of BH-30643: A novel, reversible, mutant-selective macrocyclic EGFR inhibitor invulnerable to common resistance mutations

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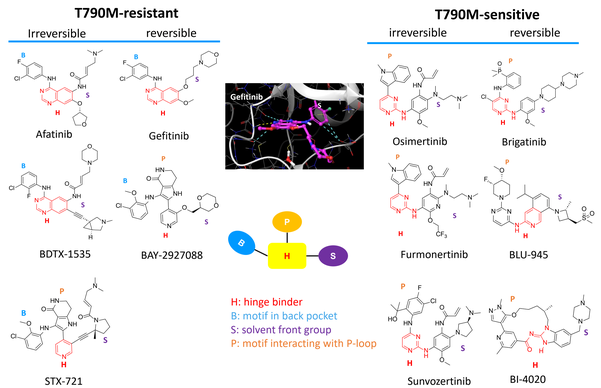
Introduction

- *EGFR*-mutant lung cancer is a global disease affecting >250,000 patients annually.
- More than one hundred different *EGFR* mutations have been reported in non-small cell lung cancer (NSCLC), falling into three established groups:
  - *EGFR* classical mutations (~70%), including exon 19 deletion (ex19del) and L858R
  - *EGFR* exon 20 insertion (ex20ins) mutations (~9%)
  - *EGFR* atypical mutations (~21%), including all other mutations
- Outcomes on EGFR tyrosine kinase inhibitor (TKI) treatment fall short of the durable benefit now observed with next-generation targeted therapies in *ALK* and *ROS1*-driven NSCLC.
- First-generation (e.g. gefitinib) and second-generation (e.g. afatinib) EGFR TKIs share a common anilinoquinazoline chemical scaffold which is vulnerable to T790M gatekeeper resistance.
- Third-generation (e.g. osimertinib) irreversible EGFR TKIs were discovered through high throughput screening, followed by lead optimization to address T790M-mediated resistance; these TKIs employ a less potent pyrimidine scaffold and obtain potency through introduction of irreversible binding at C797, which is vulnerable to C797X resistance.
- No EGFR TKI has demonstrated activity across the full spectrum of *EGFR* mutations, resulting in a diversity of treatment approaches for specific EGFR driver and resistance alterations.
- Therefore, there remains an unmet need for an EGFR TKI which can target the full spectrum of *EGFR* mutations in NSCLC with good selectivity over wildtype (WT) EGFR and prevent the emergence of resistance mutations.

Classification of EGFR TKIs Based on Binding Mode

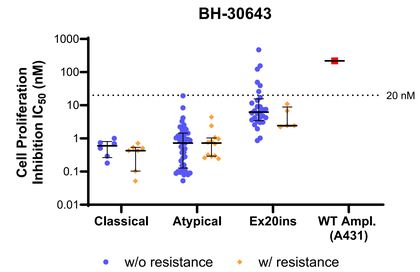
- We reviewed the chemical structures of 30+ existing EGFR TKIs in pursuit of a novel chemical scaffold that could offer a more durable treatment response.



- Across these >30 different EGFR TKIs, the key elements for binding with the EGFR protein include hinge binder (H), back pocket motif (B), P-loop interaction motif (P) and solvent front motif (S).
- EGFR inhibitors can be classified into T790M-resistance and T790M sensitive groups, based on their binding motifs at the ATP binding pocket of EGFR kinase domain.
- All the inhibitors with a motif extending into the back pocket (BHS, BHPS) will be vulnerable to resistance via the T790M gatekeeper mutation, which can limit duration of treatment benefit when EGFR T790M subclone evolving as a dominant resistance clone.
- In contrast, T790M-sensitive irreversible inhibitors (3rd generation TKIs) have no back pocket (B) motif; instead, these leverage a motif with strong P-loop (P) interaction and an acrylamide-based covalent interaction with C797, leaving these TKIs vulnerable to C797X-based resistance.
- The reversible EGFR/ALK inhibitor brigatinib shares a similar binding mode with osimertinib but only moderate potency, suggesting the pyrimidine scaffold itself (in the absence of a covalent bond) has relative weak interaction with EGFR protein.
- Some emergent investigational agents (e.g. BDTX-1535, BAY-2827088, and STX-721) leverage a back-pocket motif (B) similar to predecessor molecules, which may make them vulnerable to T790M or C797S mediated resistance mutations.
- Other investigational or preclinical agents (e.g. BLU-945 and BI-4020) explore new chemical scaffolds and leverage a P-loop interaction (P) to achieve potency against resistance mutations, yet such scaffolds have shown limitations against a full spectrum of EGFR activating mutations.
- These structural similarities are apparent on review of established and putative structures based on patent reviews of a wide diversity of EGFR TKIs:

BHS Irreversible	BHS Reversible	BHPS Irreversible	BHPS Reversible	HPS Irreversible	HPS Reversible
Afatinib	Gefitinib	STX-721	BAY2927088	Osimertinib	Brigatinib
Dacomitinib	Erlotinib	Zipalertinib		Olmutinib	BBT-176
Poziotinib	Vandetanib	ORIC-114		Almonertinib	JIN-A02
Pelitinib	Icotinib			Mobocertinib	BBT-207
Canertinib	BLU-701			Rociletinib	TRX-221
BDTX-1535	BLU-525			Naquotinib	
				Avitinib	Newer efforts:
				Nazartinib	BLU-945
				Sunvozertinib	THE-349
				Furmonertinib	

BH-30643: A Reversible, Mutant Selective OMNI-EGFR Inhibitor



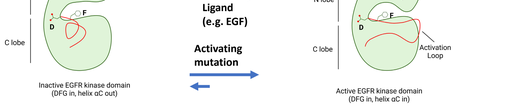
- BH-30643 was discovered to be super potent against *EGFR* classical, atypical and ex20ins mutations with good selectivity over WT EGFR.
- BH-30643 was equally potent against primary and primary/resistance (T790M or C797S) compound mutations.
- BH-30643 has slow off rate and long residence time against EGFR ex19del mutant.

Compound ID	Kinase (0.1 nM)	k_{on} (1/(M•sec))	k_{off} (1/sec)	τ (min)[a]	K_d (nM)[b]	PC9 cell (*EGFR* ex19del) pEGFR IC_{50} (nM)
BH-30643	EGFR (ERBB1) E746_A750del	9.41 X 10^5	9.94 10^{-5}	**167.6**	0.106	**0.69**
Gefitinib	EGFR (ERBB1) E746_A750del	3.67 X 10^7	4.72 X 10^{-4}	35.3	0.013	5.07
BLU-945	EGFR (ERBB1) E746_A750del	6.36 X 10^5	4.20 X 10^{-3}	4.0	6.60	427
BAY2927088	EGFR (ERBB1) E746_A750del	7.41 X 10^6	1.36 X 10^{-4}	122.8	0.018	1.20

[a] $\tau = 1 / k_{off}$ [b] $K_d = k_{off} / k_{on}$

Design Principle: Targeting EGFR Active Conformation

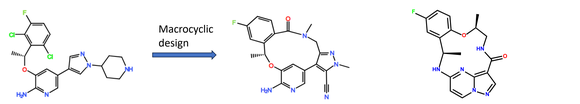
EGFR mutations drive mutant EGFR to the active conformation



- In normal physiologic conditions, EGFR exists dominantly in its inactive conformation and shifts to the active conformation only after ligand stimulation.
- Activating *EGFR* mutations (classical, atypical and ex20ins) and resistance mutations push the EGFR protein into an active conformation with a more compact ATP pocket and a stronger interaction with ATP.
- Targeting the EGFR active conformation led to the design of a compact molecule at ATP binding pocket which achieves potency against all EGFR mutations (classical, atypical, ex20ins, and compound resistance) with selectivity over WT EGFR.

Macrocyclic Design Enables to Achieve Super Potency and Selectivity

- A macrocyclic design approach in the development of next-generation ALK and ROS1 TKIs has enabled super-potency and prolonged progression free survival (PFS) while overcoming resistance mutations.

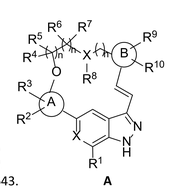


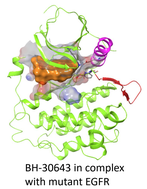
Crizotinib
ALK cell potency: 87 nM
ROS1 cell potency: 12 nM
PFS: 10.9 months in *ALK*+ NSCLC
PFS: 19.2 months in *ROS1*+ NSCLC

Lorlatinib
ALK Cell Potency: 2.4 nM
PFS: >60 months in *ALK*+ NSCLC

Repotrectinib
ROS1 Cell Potency: 0.2 nM
PFS: 35.7 months in *ROS1*+ NSCLC

- A novel macrocyclic scaffold (A) was designed based on the structural features and characteristics of the active conformation of mutant EGFRs; a comprehensive lead optimization was carried out to identify the clinical candidate BH-30643.
- Kinase screen identified only 4 hits with >90% enzymatic kinase activity inhibition out of 372 wild type human kinases at 100 nM of BH-30643.

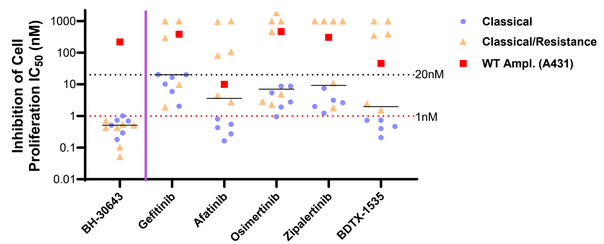




BH-30643 in complex with mutant EGFR

BH-30643 Potently Inhibits EGFR Classical/Resistance Mutations

- Approved and investigational EGFR TKIs have recurring liabilities including T790M or C797S resistance, inadequate potency, or narrow therapeutic window leading to WT EGFR toxicity.

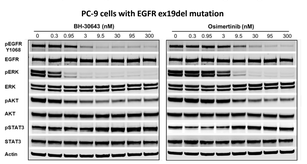


Cell Proliferation IC_{50} (nM)												
Compound	PC-9 (EGFR d746- 750)	HCC827 (EGFR d746-750)	Ba/F3 EGFR d746-750 /T790M	Ba/F3 EGFR d746-750 /C797S	Ba/F3 EGFR d746-750 /T790M/C797S	Il-18 (EGFR L858R)	H3225 (EGFR L858R)	H1975 (EGFR L858R/ T790M)	Ba/F3 EGFR L858R/C797S	BaF3 EGFR L858R /T790M/C797S	A431 (EGFR WT Amp)	Ba/F3 +IL3
BH-30643	**0.74**	**0.29**	**0.43**	**0.10**	**0.43**	**0.82**	**0.37**	**0.72**	**0.05**	**0.54**	**217**	**4450**
Osimertinib	5.48	2.74	2.85	>1000	>1000	8.72	5.78	4.86	462	>1000	469	3919
Afatinib	0.75	0.43	106	2.80	954	0.61	0.29	84.4	4.39	>1000	9.94	3532
Gefitinib	20.1	5.90	>1000	1.92	>1000	16.8	2.26	>1000	9.82	>1000	385	>10000

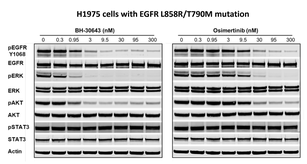
- BH-30643 is significantly more potent than osimertinib in cells with classical or classical/T790M compound mutations and overcomes the C797S resistance mutation with or without T790M.
- BH-30643 potently inhibited pEGFR in cell lines with EGFR classical mutation or classical/resistance mutations (compound with T790M, C797S, or T790M/C797S), and downstream signaling.

pEGFR-Y1068 by TR-FRET IC_{50} (nM)				
Compound	PC-9 (ex19del)	H197S (L858R/ T790M)	Ba/F3 EGFR (L858R/ C797S)	Ba/F3 EGFR (L858R/T790M/ C797S)
BH-30643	**0.69**	**1.10**	**3.04**	**3.35**
Osimertinib	8.59	11.5	>1000	>1000
Afatinib	0.64	9.49	9.75	>1000
Gefitinib	5.07	>10000	8.24	>1000

PC-9 cells with EGFR ex19del mutation

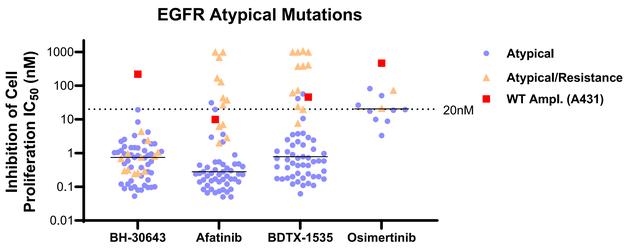


H1975 cells with EGFR L858R/T790M mutation



BH-30643 Potently Inhibits EGFR Atypical/Resistance Mutations

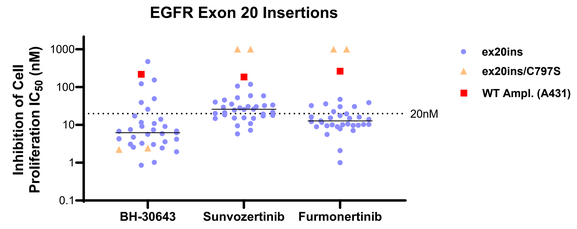
- Approved and investigational EGFR TKIs targeting atypical mutations share recurring liabilities including a narrow therapeutic window leading to wildtype EGFR toxicity, vulnerability to resistance mutations, or inadequate potency.



Cell Proliferation IC_{50} in Engineered Ba/F3 Cells with EGFR Mutants (nM)													
Compound	E709K	G719C	G719S	G724S	L747P	S768I	T790M	L861Q	G719A/ S768I	G719A/ L861Q	G719A /T790M	L858R/ E709A	T790M/ C797S	L858R/ K860I	L858R/ T854A	L718V /L858R /T790M
BH-30643	**1.19**	**0.08**	**0.37**	**5.90**	**1.56**	**2.22**	**1.57**	**0.85**	**1.19**	**0.57**	**0.25**	**0.46**	**0.85**	**0.20**	**2.45**	**0.97**
Afatinib	0.14	0.16	0.08	0.26	0.38	0.36	27.9	0.21	0.47	0.44	2.01	0.21	660	0.10	0.19	170
BDTX-1535	1.13	0.12	0.18	0.93	2.49	3.54	779	0.24	1.09	0.70	24.1	0.50	>1000	0.22	0.75	>1000
Furmonertinib	8.85	1.80	4.45	5.53	9.65	22.9	6.29	1.20	10.2	3.69	4.17	1.46	>100	0.63	0.55	6.64
Osimertinib	19.4	3.31	17.6	78.5	8.80	18.7	16.6	9.94	81.2	50.6	21.4	26.4	560	13.2	4.11	72.9

BH-30643 Potently Inhibits EGFR Ex20ins/Resistance Mutations

- No EGFR TKI is approved for EGFR exon 20 ins; investigational TKIs have liabilities including C797S resistance mutation and/or suboptimal therapeutic window.



Cell Proliferation IC_{50} in Engineered Ba/F3 Cells with EGFR ex20ins Mutant (nM)						
Compound	A763_Y764insFQEA	V769_D770insASV	D770_N771insSVD	D770_N771insNPG	H773_V774insNPH	A431 (WT EGFR)
BH-30643	**1.80**	**4.31**	**39.9**	**7.40**	**87.3**	**217**
Furmonertinib	6.35	15.1	40.4	10.2	34.2	269
Sunvozertinib	14.6	17.8	50.4	16.0	41.0	186
Zipalertinib	3.18	49.7	70.0	13.7	123	288
Osimertinib	9.87	44.5	181	35.6	151	469

BH-30643 Inhibits Mutant HER2 With Limited WT HER2 Activity

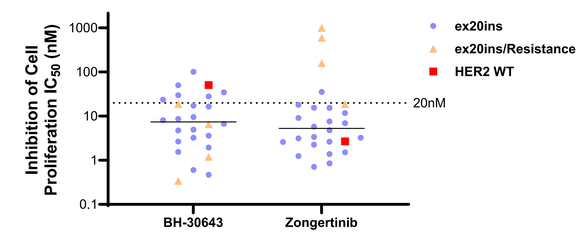
- WT HER2 inhibition is a recurring liability of EGFR TKIs leading to dose-dependent diarrhea.
- BH-30643 has moderate preclinical potency against WT HER2.

Cell Proliferation IC_{50} (nM)									
Compound	BH-30643	Mobocertinib	Afatinib	Furmonertinib	Sunvozertinib	STX-721	Osimertinib	BDTX-1535	Lazertinib	Zipalertinib
BaF3 WT HER2	43.8	0.55	1.54	2.60	6.50	8.11	9.43	37.3	134	251

- WT HER2 inhibition activity narrows therapeutic window for many historical EGFR/HER2 TKIs.
- BH-30643 targets HER2 active conformation, achieving greater potency against mutant HER2.
- Many HER2 inhibitors (e.g. zongertinib) are irreversible, target HER2 inactive conformation, are more potent against WT HER2 and are vulnerable to resistance mutations.
- BH-30643 can overcome HER2 resistance mutations with a favorable therapeutic window.

Cell Proliferation Inhibition IC_{50} in Engineered Ba/F3 Cells with HER2 WT and Mutants (nM)				
Compound	HER2 WT	HER2 A775_G776 insYVMA	HER A775_G776 insYVMA/C805S	HER2 T798M
BH-30643	**43.8**	**18.8**	**6.53**	**3.28**
BAY2927088	5.51	8.23	11.6	ND
Zongertinib	2.22	10.1	598	146
Tucatinib	15.4	114	364	ND

HER2 Exon 20 Insertions



Conclusion

- BH-30643 is a novel macrocyclic, reversible, mutant-selective EGFR TKI targeting the active conformation of mutant EGFR, with slow-off rate and long residence time.
- We observe super-potency against EGFR classical, atypical, and compound/resistance mutations in cell proliferation assays, with potency maintained against EGFR exon 20 ins and HER2 mutations.
- Selectivity is demonstrated against WT EGFR and WT HER2, with negligible preclinical off-target kinase activity.
- Based on favorable PK properties and favorable toxicology studies, IND was submitted in 2H/2024 and clinical investigation has begun.
- The international phase I/II SOLARA trial (NCT06706076), for patients with advanced NSCLC harboring EGFR or HER2 mutations, is now enrolling.

Note: All reference compounds in this poster are proxy ones from commercial vendors or made in house.

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