Design and Synthesis of Quinazoline Derivatives as Novel, Potent Multi Acting HDAC and Receptor Tyrosine Kinase Inhibitors for the Treatment of Cancer

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Introduction

HDACs and receptor tyrosine kinases (RTKs) such as EGFR and Her2 are validated cancer targets. A multi-targeted HDAC and RTK inhibitor may offer more therapeutic benefits in the treatment of cancer in comparison to single-acting agents due to potential synergistic effects between HDAC and RTK inhibition. By incorporating HDAC inhibitory functionality into the RTK inhibitor pharmacophore, a novel quinazoline series of potent multi acting compounds were designed and synthesized. Through extensive structural modifications and SAR studies, CUDC-101, one of the lead molecules in the series, was prepared and demonstrated very potent HDAC, EGFR and Her2 inhibition. This drug candidate is currently being prepared for an IND filing and is anticipated to enter a human clinical Phase I trial for solid tumors in the first half of 2008.

Strategies and Compound Design

1. Structure requirements

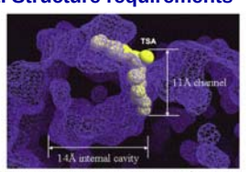


Figure 1. Surface representation of the 11 Å channel and the 14 Å internal cavity of the HDLP–TSA complex. TSA displayed as yellow CPK model.[6]

(J. Med. Chem, 2005, 48, 6936-6947)

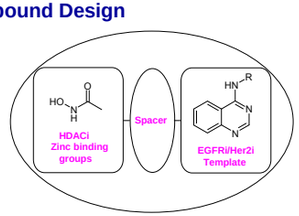
- Aliphatic chain has multiple contacts with hydrophobic residues in the tube-like, 11 A deep channel (channel afterwards) which leads to the active site
- Hydroxamic acid interacts with Zn+ at the active site to disrupt the enzyme activity of HDAC
- Large hydrophobic region binds to the hydrophobic part of the enzyme
- A 14Å long side pocket (internal cavity afterward) may act as a second binding site

FIG. 6. **Stereoview of the inhibitor binding site and nearby residues from EGFRK/erlotinib.** *Dashed line* indicates an H-bond from the Met^{769} amide nitrogen to erlotinib. *The light blue sphere* is a water molecule. The electron density for the side chains of Cys^{773} and Asp^{776} was modeled using two conformers, but only one is depicted for clarity.

[1] J. Bio. Chem. 2002, 48, 46265-72
2) Current Signal Transduction Therapy, 2006, 1, 67-97]

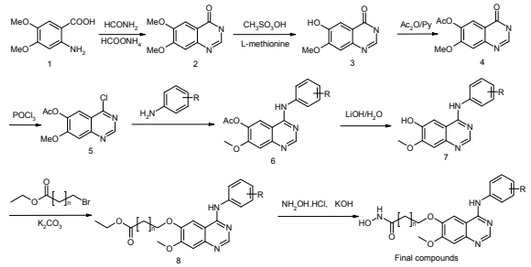
- Quinazoline occupies the adenine region of the ATP binding site and forming two hydrogen bonds with N1 and N3
- Phenyl-amino group occupies the hydrophobic pocket
- Methoxy-ethoxy groups stick out of the receptor to ensure proper solubility and bioavailability

2. Compound Design



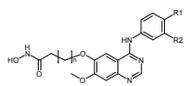
- Template represents EGFR/Her2 pharmacophore
- Hydroxamic acid represents HDAC pharmacophore
- Spacer serves as a bridge to properly display EGFR/Her2 and HDAC pharmacophores
- Our strategy was to design and synthesize compounds that meet the structural requirements of HDAC and EGFR/Her2 inhibition and therefore possess potent multi-targeted activities
- Potential synergistic effect between inhibition of individual target may provide great therapeutic benefits in the treatment of cancer

Representative Synthesis



(Authors thank the contributions of this synthetic work provided by Curis FTE team at Shanghai ChemPartner Co., Ltd, Shanghai, China)

SAR of Chain Length and Ring Substitution

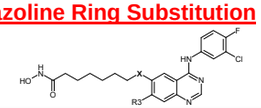


Compound #	n	R1	R2	IC50 (nM) in Enzyme Assays		
				HDAC	EGFR	HER2
1	0	F	Cl	>100000	1.6	ND
2	1	F	Cl	40900.0	7.1	ND
3	2	F	Cl	>100000	7.8	ND
4	3	F	Cl	551.0	9.4	ND
5	3	H	≡CH	421.0	15.1	ND
6	4	F	Cl	31.0	3.4	ND
7	4	H	≡CH	13.2	4.6	157.0
8	5	F	Cl	6.5	3.1	23.4
9	5	H	≡CH	4.6	3.0	25.1
SAHA				40.0	N/A	N/A
Erlotinib				N/A	48.0	134.5
Lapatinib				N/A	11.2	10.2

ND: not determined

- Extension of the length of the hydroxamic acid side chain increases HDAC inhibitory activity while EGFR inhibition is largely unchanged
- Either 3-Cl, 4-F or 3-ethynyl substituted aniline gives similar inhibitory activity against HDAC, EGFR or Her2

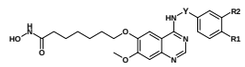
SAR of Quinazoline Ring Substitution



Compound #	X	R3	IC50 (nM) in Enzyme Assays		
			HDAC	EGFR	HER2
8	O	CH_3O-	6.5	3.1	23.4
10	O	H	4.4	84.2	320.5
11	O	$CH_3OCH_2CH_2O$-	8.4	10.4	107.2
12	-CONH-	CH_3O-	15.3	2.1	76.2
13	S	CH_3O-	39.6	1.99	7.21
14	SO_2	CH_3O-	121.7	15.4	ND

- R3: CH_3O or $CH_3OCH_2CH_2O$ or H gives similar inhibitory activity against HDAC while potency of EGFR or Her2 inhibition has a order of CH3O- > CH3OCH2CH2O- > H
- X: HDAC inhibition: O > CONH > S > SO2
  EGFR inhibition: O ~ CONH ~ S > SO2
  Her2 inhibition: S > O > CONH

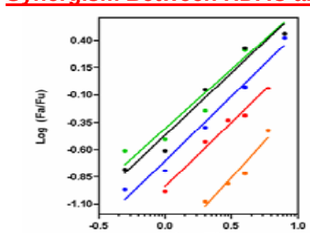
SAR of Right Side Ring Variations



Compound #	Y	R1	R2	IC50 (nM) in Enzyme Assays		
				HDAC	EGFR	HER2
8	direct bond	F	Cl	6.5	3.1	23.4
15	CH2	F	Cl	11.3	72.0	1073.0
16	CH2	H	H	4.2	99.4	676.0
17	CH2	F	H	5.6	268.0	ND
18	(S)-CHCH3	H	H	25.7	5077.0	ND
19	(R)-CHCH3	H	H	98.4	12.8	317.8
20	(R)-CHCH3	F	H	165.3	125.6	ND
21	(R)-CHCH3	Cl	H	188.0	614.0	ND
22	direct bond		Cl	58.1	9.08	17.5

- Right side benzylamine resulted in lower activity than the aniline ring in HDAC, EGFR and Her2 inhibition
- R isomer is ~4 fold more potent in HDAC inhibition and ~400 fold more potent in EGFR inhibition compared with its S isomer
- In benzylamine series, 4-F or 4-Cl or 3-Cl, 4-F analog is less potent than the corresponding 4-H analog in both HDAC and EGFR inhibition
- Large R1 reduces HDAC and EGFR inhibition but does not affect Her2

Synergism Between HDAC and EGFR inhibition



- Addition: CI=1
- Synergy: CI<1
- Antagonism: CI>1

Drugs	Ratio	CI
EGFRi : HDACi	1:1	0.277
EGFRi : HDACi	3:1	0.37
EGFRi : HDACi	1:3	0.356
HDACi		
EGFRi		

- Reference compounds that inhibit EGFR or HDAC were combined in various ratios to assess possible synergistic effects
- Combination Index (CI) values ≤1 indicate synergy; the lower the CI, the greater the degree of synergy
- Potent synergism was observed over a 9-fold range of ratios, indicating that very specific HDACi and EGFRi ratios are not required for strong biological activity when incorporated into a single small molecule

CUDC-101 is a Potent and Selective Multi Acting Inhibitor

- **Potency:**
  HDAC inhibition: 5-10 fold more potent than SAHA
  EGFR inhibition: 10-20 fold more potent than erlotinib
  Her2 inhibition: 5-10 fold more potent than erlotinib and similar to lapatinib
- **Selectivity:**
  72 kinases screened
  Weak inhibitor of VEGFR2, Lyn, Abl-1, FGFR2, Flt3, Lck and Ret kinases (IC50 values are within the range of 1-5 μM)
  Inhibition of other kinases is less than 50% at 10 μM

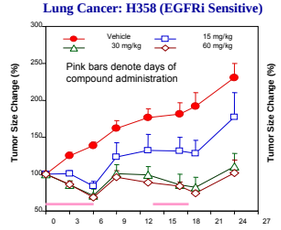
CUDC-101 Effectively Inhibits Proliferation of Human Cancer Cell Lines

Cancer Type	Cell Line	SAHA	Erlotinib	SAHA & Erlotinib	Lapatinib	SAHA & Lapatinib	CUDC-101	Improvement vs. S/E	In Potency vs. S/L
NSCLC	H1975	4.70	>10	4.00			0.50	8x	
NSCLC	H1703	4.70	9.00	1.80			0.20	9x	
NSCLC	Calu-6	1.80	3.20	1.00			0.20	5x	
NSCLC	HCC827	1.80	7.50	2.30			0.60	4x	
NSCLC	H358	2.50	6.00	1.10			0.40	3x	
NSCLC	A549	2.60	>20	1.40			0.50	3x	
NSCLC	H292	1.10	1.30	0.60			0.20	3x	
NSCLC	H460	1.70	8.20	1.40			0.70	2x	
NSCLC	H2122	7.50	1.00	0.40			0.30	1x	
Pancreatic	HPAC	1.57	10.87	0.77			0.08	9x	
Pancreatic	MiaPaCa	1.70	15.80	1.70			0.29	6x	
Pancreatic	CFPAC	7.70	14.90	3.20			0.56	6x	
Pancreatic	CaPan1	7.30	>20	4.40			0.80	6x	
Pancreatic	PANC	4.30	>20	3.40			0.66	5x	
Pancreatic	BxPC3	2.70	7.60	1.00			0.27	4x	
Prostate	22RV1	1.70	>20	1.20			0.06	12x	
Prostate	PC-3	4.80	>20	3.00			0.61	5x	
Renal	Caki-1	0.11	0.06	0.12			0.04	3x	
Epidermoid	A431	1.00	2.33	0.54			0.23	2x	
Breast	MCF-7	2.80	>20	2.70	6.60	2.60	0.55	5x	5x
Breast	MDA-MB-231	2.11	>20	2.00	5.40	1.80	0.10	20x	18x
Breast	MDA-MB-468	5.01	11.40	1.60	3.60	1.10	0.21	8x	5x
Breast	BT-474	0.48	1.90	0.41	0.06	0.04	0.07	6x	<1x
Breast	Sk-Br-3	1.19	1.56	0.90	0.04	0.05	0.04	23x	1x
Colon	HCT-116	1.15	18.60	1.37	6.40	1.18	0.10	14x	12x
Colon	WiDr	0.61	16.10	0.55	1.64	0.58	0.07	8x	8x
Colon	HT-29	1.29	>20	1.20	5.80	1.10	0.25	5x	4x
Liver	Sk-Hep-1	3.46	10.37	3.97	5.30	1.50	0.22	18x	7x
Liver	HepG2	1.66	>20	1.95	6.27	1.23	0.13	15x	10x
Liver	Hep3B2	2.44	>20	2.64	5.49	2.36	0.23	12x	10x

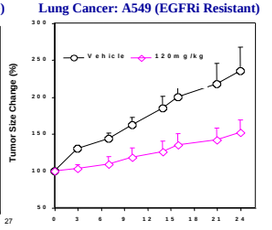
Note: Numbers indicated IC50 in μM

CUDC-101 is Efficacious in Xenograft Models

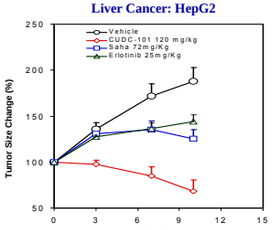
Lung Cancer: H358 (EGFRi Sensitive)



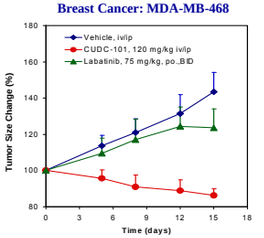
Lung Cancer: A549 (EGFRi Resistant)



Liver Cancer: HepG2



Breast Cancer: MDA-MB-468



Conclusion

- Extensive structure and activity relationship (SAR) has been investigated
- CUDC-101 has been identified as a potent HDAC, EGFR and Her2 inhibitor with broad range of anti-proliferation activity in vitro and high efficacy in xenograft models
- Synergistic effects between HDAC and EGFR inhibition suggests CUDC-101 may have the potential to overcome limitations observed in the treatment of heterogeneous and drug-resistant tumors by traditional single acting inhibitors
- CUDC-101 displays a favorable safety profile (data not shown) and is a well qualified candidate for clinical development and is anticipated to enter Phase I in the first half of 2008