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INFO SHEET

Leveraging atropisomerism for highly selective small molecule kinase inhibitors

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Type(s) of relationship sought: Licensing, Funding for sponsored research, collaborators for clinical validation studies, commercialization, and/or investment

The problem

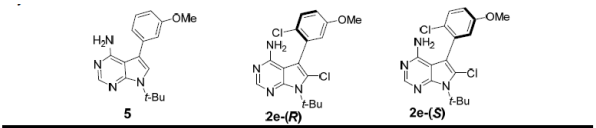
Aberrant kinase activity has been implicated in numerous diseases, including cancer. Kinase inhibitors serve as chemical probes to elucidate the role of a kinase or signaling pathways in cellular processes or disease. A large degree of active site conservation throughout the kinase family causes most kinase inhibitors to possess inhibition activities towards multiple kinases and off-target kinase inhibition affecting unintended signaling pathways. Despite recent attempts to increase small molecule selectivity towards a specific kinase using 'selectivity filters' that take advantage of unusual features in a kinase active site, a general selectivity filter has remained elusive as by design they rely on rare occurrences in an active site. Atropisomerism is a form of chirality that arises from hindered rotation around an axis that renders the rotational isomers enantiomers. Many biologically active small molecules possess little hindrance to rotation and exist as a rapidly interconverting mixture of atropisomers yet bind to their respective biological targets in an atropisomer-specific manner. Since atropisomers can display drastically different pharmacological profiles, it can cause serious complications in drug development, especially when a compound possesses an intermediate stability, and can racemize over the length of the experiment.

SDSU Solution

The Gustafson Lab has is developing compounds with increased kinase selectivity, and methods to accomplish this. The team has synthesized and tested pyrrolopyrimidine-based kinase inhibitor that comprises an atropisomerism rotational blocking moiety on a rotatable phenyl moiety of the parent kinase inhibitor, which conformationally stabilizes the atropisomer having modulated kinase selectivity.

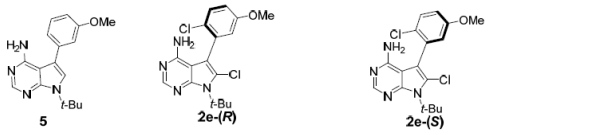
An (*R*)-atropisomer selective for rearranged during transfection (RET) kinase, YES kinase, or a combination thereof has been tested for the growth inhibition of the cells of gastrointestinal stromal tumors (GIST), medullary thyroid cancer, ER-positive breast cancer, or non-small cell lung cancer, melanoma, breast cancer, or rhabdomyosarcoma. Also, an (*S*)-atropisomer selective for SRC kinase, ABL kinase, YES kinase, or a combination thereof has been tested against the growth inhibition of cancer cells that cause breast cancer, colon cancer, prostate cancer, chronic myeloid leukemia, melanoma, breast cancer, or rhabdomyosarcoma.

The team has generated selective kinase inhibitors wherein an (S)-atropisomer or (R)-atropisomer is selective for a kinase in terms of inhibition/selectivity **(Table 1)** and differential selectivity between atropisomers has been studied for various kinases **(Table 2)**.



Kinase[a]	% inhibition 200 nM[b]	% inhibition 1000 nM[b]	% inhibition 1000 nM[b]	% inhibition 5000 nM[b]	% inhibition 1000 nM[b]	% inhibition 5000 nM[b]	S/R (5000 nM)[d]
Abl	37%	78%	11%	26%	36%	67%	2.57
Alk	11%	23%	6%	18%	5%	7%	0.38
Blk	38%	74%	3%	32%	21%	47%	1.46
BTK	62%	90%	10%	29%	18%	54%	1.86
CSK	36%	65%	21%	35%	19%	45%	1.28
EGFR	28%	62%	-4%	0%	10%	21%	---
Her-2	4%	16%	-2%	0%	-7%	0%	---
Fgr	69%	92%	45%	77%	58%	81%	1.05
Fyn	56%	84%	19%	44%	26%	56%	1.27
Hck	59%	80%	24%	40%	32%	50%	1.25
Kit	8%	27%	5%	26%	18%	36%	1.38
Lck	32%	79%	23%	51%	26%	54%	1.05
Lyn	58%	85%	19%	57%	36%	75%	1.31
pdgfr-a	11%	34%	3%	8%	3%	13%	1.63
pdgfr-b	6%	19%	4%	13%	13%	16%	1.23
Ret	59%	89%	35%	73%	14%	32%	0.44
Yes	63%	90%	53%	79%	53%	77%	0.97
S(40%)[c]	**0.44**	**0.72**	**0.11**	**0.44**	**0.16**	**0.61**	**----**

Table 1: Percent inhibition of 2e atropisomers and 'parent' compound 5 across a panel of tyrosine kinases at 2 concentrations



Kinase	5 IC50	IC50 of 5 Kinase/ IC50 of 5 Yes	2e-(R) IC50	IC50 of 2e-(R) Kinase/ IC50 of 2e-(R) Yes	2e-(S) IC50	IC50 of 2e-(S) Kinase/ IC50 of 2e-(S) Yes
Src	151+/-9 nM[b]	1.64	5570+/-907 nM[b]	6.22	1193+/-170 nM[b]	1.64
EGFR	641+/-54 nM[b]	6.96	>10,000 nM[c]	>10.0	>10,000 nM[c]	>10.0
Yes	92+/-11 nM[b]	1	895+/-90 nM[b]	1	727+/-177 nM[b]	1
Ret	128+/-3 nM[b]	1.4	1857+/482nM[b]	2.07	7659+/-754 nM[b]	10.53
Abl	244.5+/-19 nM[c]	2.65	>10,000 nM[c]	10.0	1432+/-210 nM[c]	1.96

[a] Data obtained at Life Technologies using the Z'-LYTE kinase inhibition platform. Error is Standard Deviation. [b] IC50 determined in triplicate. [c] IC50 determined in duplicate.

Table 2: IC_{50} data of atropisomeric kinases inhibitors

The team has obtained drastic improvements in potency and selectivity by strategically adding five nonhydrogen atoms to a highly promiscuous kinase inhibitor scaffold (PP1) **(Figure 1)**.

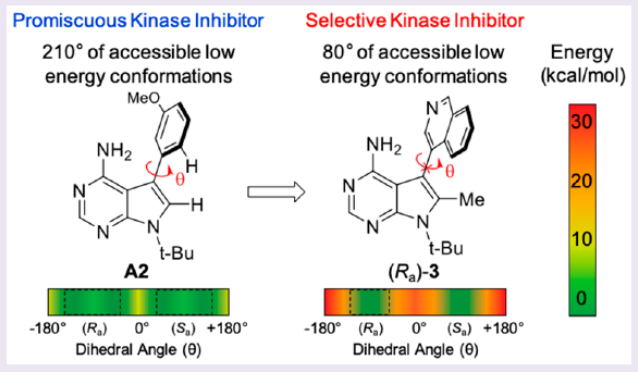


Figure 1. Limited access to dihedral angles as a consequence of atropisomerism

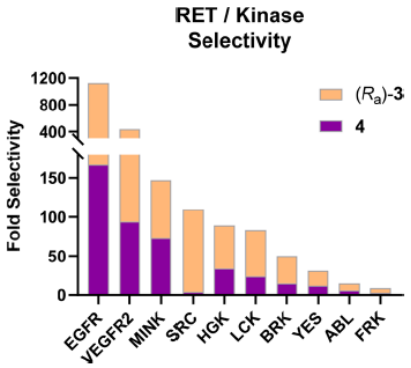


Figure 2. Graphical comparison of RET selectivity for (Ra)-3 and 4 sorted by fold selectivity

The lead compound, *(Ra)-3* displays 2−3 orders of magnitude selectivity for RET over epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor 2 (VEGFR2), kinases whose off-target inhibition leads to well characterized adverse events in patients **(Figure 2)**, displays good antiproliferative activities in RET-driven models of various cancers **(Table 3)** and has no observable effects against cell lines that are not RET-dependent. Additionally, (R_a)-3 represents a **first-in-class, highly mutant-selective EGFR inhibitor** that displays cellular activity as a single agent toward the triple mutant-driven cell line. (R_a)-3 spares wild type EGFR but potently inhibits several EGFR mutants, including the vaunted L858R/T790M/C797S triple mutant **(Table 4)**.

cell line	(R_a)-3	(S_a)-3	4	Vandetanib	BLU-667[28]
LC-2/AD (CCDC6-RET)	2.81	>10	4.3	1.47	0.004[b]
TT (RET C634W)	1.45	>10	2.59	1.01	0.015[b]
ED-MCF7 (overexpressed WT RET)	1.15	>10	1.31	0.25	
BT474 (+ER, PR and HER2)	>10	>10	>10	5.91	>10
H292 (overexpressed WT EGFR)	>10	>10	>10	1.14	>10
cell line	(R_a)-3	(S_a)-3	4	Osimertinib	Brigatinib
H292 (overexpressed WT EGFR)	>10	>10	>10	3.75	2.76
H1975 (EGFR L858R/T790M)	4.3	>10	5.0	0.343	0.890
Ba/F3 (EGFR L858R)	2.27	>10	2.60	0.015	0.385
Ba/F3 (EGFR L858R/T790M)	5.87	>10	8.38	0.064	0.527
Ba/F3 (EGFR L858R/T790M/C797S)	5.42	>10	6.20	3.18	0.882
Ba/F3 (empty vector)	>10	>10	>10	2.76	2.10

[a]Cell growth GI_{50} values are reported in micromolar and were measured in triplicate. [b]Data taken from ref 28.

Table 3. Anti-Proliferative Effect of Inhibitors

kinase K_i (nM)	(R_a)-3	4	Osimertinib[61,63]	Brigatinib[42,62]
EGFR WT:	1623	721	8.4	67
EGFR L858R:	77	111.5	6.1	1.5
EGFR T790M:	16.5	29.3	2.1	
EGFR L858R T790M:	15.8	17.7	4.2	29
EGFR L858R T790M C797S:	53	69.5	139	2.79

[a]K_i values are reported in nanomolar and were measured in duplicate.

Table 4. Inhibitor activity on WT EGFR and Mutants

Ibrutinib is a multi-kinase inhibitor that is used for treatment of Burton Tyrosine Kinase (BTK) driven cancers; however, shows off-target inhibition. The team has synthesized, characterized, and tested conformationally-controlled Ibrutinib analogs and finds that these are more selective than Ibrutinib in that the former are preferred by BTK but not the other off-target kinases **(Figure 3)**. They have employed molecular docking studies, analyzed the inhibitors' conformations, and synthesized an enantiomer (*S* enantiomer) where the bond rotation directs an acrylamide moiety toward Cys481 compared to enantiomer (*R*) in which a bond rotation positions the acrylamide out of Cys481's range **(Figure 4)**.

Out of the atropisomeric analogs tested, (R_a, *S*) possessed the highest affinity towards BTK, 10x more potent than the diastereomer (R_a, *R*), which suggests that the piperidine conformation plays a major role in binding affinity. While less potent than Ibrutinib, (R_a, *S*) displayed increased selectivity for BTK over each of the tested kinases **(Figure 5)**. The compound has been tested for growth inhibition of cancer cells of the breast, lung, pancreas, prostate, colon, blood, or thyroid.

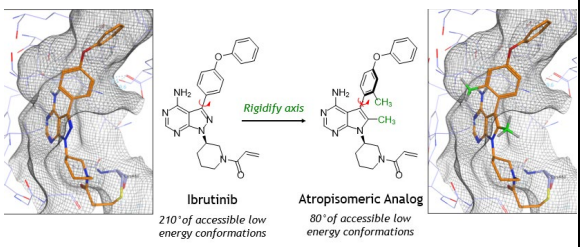


Figure 3. Atropisomerism as a Selectivity Filter

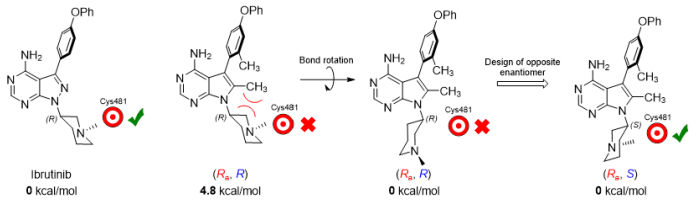
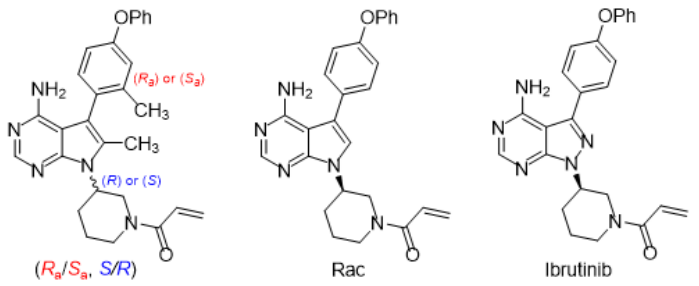


Figure 4. Conformational Control of Piperidine



Kinase IC_{50} (nM)	(R_a, S)	(R_a, R)	(S_a, R)	(S_a, S)	Rac	Ibrutinib[ref4]
BTK	425	3307	>10000	3923	0.47	1.5
HER2	>10000	>10000	>10000	>10000	451	6.4
ITK	>10000	>10000	>10000	>10000	-	4.9
BLK	187	1814	9964	1732	0.29	0.1

Figure 5: In vitro kinase assays

Value proposition

While RET TK inhibitors (TKIs) are used/being developed to treat certain cancers, the effect of mutations in the RET kinase domain on drug sensitivity is deemed largely uncharacterized. Despite the third-generation EGFR tyrosine kinase inhibitors (osimertinib and Lazertinib) being available, their efficacy to treat cancer is compromised by additional acquired mutations, including C797S. An urgent unmet medical need exists for next-generation EGFR TKIs for selective inhibition of L858R/T790M/C797S EGFR triple mutations. Use of BTK inhibitor ibrutinib is associated with drug resistance and intolerable side effects in patients, e.g., those with chronic lymphocytic leukemia (CLL).

The Gustafson Lab's novel approach leverages atropisomerism to restrict the accessible low-energy dihedral conformations available to a promiscuous kinase inhibitor and to achieve highly selective and potent inhibitors of oncogenic targets. Different members of highly conserved families of enzymes such as kinases can prefer different atropisomer conformations of the same inhibitor, and this can be harnessed to modulate inhibitor promiscuity.

Applications

The portfolio of kinase inhibitors is indicated primarily in oncology with applicability for the conditions below.

Type of cancer	**Reported mutations**
NSCLC, thyroid cancer, Tamoxifen-resistant breast cancer, neuroblastoma	RET kinase
NSCLC, breast cancer	Drug-resistant (non-druggable) EGFR mutants including L858R/T790M/C797S EGFR
Mantle cell lymphoma (MCL), chronic lymphocytic leukemia (CLL)	BTK

Stage of Development

The Gustafson lab has programs on EGFR mutants (focusing on the L858R/T790M/C797S triple mutant). The lead compounds' selectivity (2-3 orders of magnitude of) for the major EGFR mutants over wild type has been

verified in cell free assays, and cellular assays. Plans to study the lead compounds in mice are underway. A highly selective RET inhibitor (exceeding the selectivity of the BLU and LOXO molecules with comparable RET potencies) has been verified in biochemical assays and cellular assays (mid-nanomolar EC50s). In addition, BTK compounds have been tested in *in vitro* assays. Ibrutinib analogs are more selective than Ibrutinib. Before moving the inhibitors down the pipeline to cancer cell line assays and mice experiments, the team would like to generate more analogs with improved potency.

Patents and Publications:

U.S. Patent Nos. 11,345,707 and 10,550,124 – "Atropisomerism for Increased Kinase Inhibitor Selectivity"
EU Patent Application No. 16836030.3 – "Exploiting atropisomerism to increase the selectivity of kinase inhibitors"
U.S. Patent No. 10,934,300 – "Atropisomerism for Enhanced Kinase Inhibitor Selectivity"
EU Patent Application No. 18821459.7 - "Atropisomeric Pyrrolopyrimidine Based KinaseInhibitors With Improved Selectivity Towards RET Kinase"
U.S. Patent Application No. 17/619,688 - "Selective BTK Irreversible Inhibitors"

"Leveraging Atropisomerism to Obtain a Selective Inhibitor of RET Kinase with Secondary Activities toward EGFR Mutants," Sean T. Toenjes, Valeria Garcia, Sean M. Maddox, Gregory A. Dawson, Maria A. Ortiz, F. Javier Piedrafita, and Jeffrey L. Gustafson; *ACS Chem Biol*, 2019, 14, 1930−1939.

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