# Synthesis and Comparative study of a Library of Small Molecules Capable of Disrupting HIF2α Dimerization

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## Abstract:

In the past, it has been shown that tetrazolamine derivatives are capable of inhibiting the dimerization of HIF-2α, an important molecular target in the pathway of neovascularization both aberrantly and overly expressed in different cancer cell lines such as certain neuroendocrine tumors [1]. Using the molecular scaffolding of tetrazolamine ring as a base, 24 different derivatives were synthesized and tested to determine an efficacious (potent) inhibitor of HIF-2α in terms of Kd. This study, firstly, is a stepping stone for the determination of higher potency compounds targeting HIF2α transcription factor, and, secondly, more clearly defines the shape of the molecular binding site of the compounds and, finally, possibly represents the beginning stages of including a new molecular target in the treatment of metastatic cancer.

## Introduction:

Currently, there are very few drugs that target limiting the angiogenesis of solid tumors. Of note, however, Bevucizumab (Avastin ®) is a monoclonal antibody targeting VEGF used in the clinical treatment of metastatic colon cancer exemplifying the potential of using angiogenesis limiting drugs in combination chemotherapy treatment of metastatic cancers and those less amenable. The interactions of this family of tetrazolamine derived compounds have shown activity inhibiting the dimerization of HIF2**α** transcription factor. The differences in the activities of the derivatives highlight the shape of the molecular binding site of the drug and how well the different halogens, both in physical size and electronic properties, interact in destabilizing the dimerization of the complex.

## Methods:

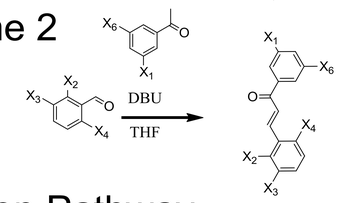
All compounds were synthesized in Tambar lab by the following synthetic schemes and separated stereochemically to pure products. These were then tested for activity in Bruick and Gardner labs. Kd were empirically derived via isothermal calorimetry while Alphascreen was used to find IC50. Two enantiomers were tested for activity in vitro by titrating amounts of active compound, inducing hypoxic conditions and performing western blot analysis to see drug effects on cell protein regulation.

## Synthetic Schemes

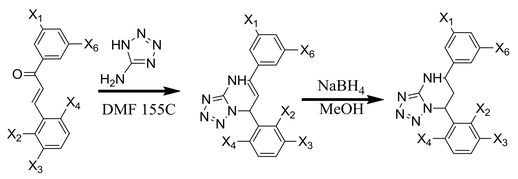
### Scheme 1



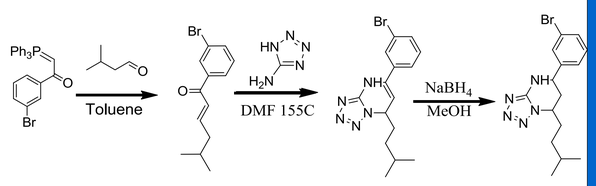

### Scheme 2




### Common Pathway




### Scheme 3

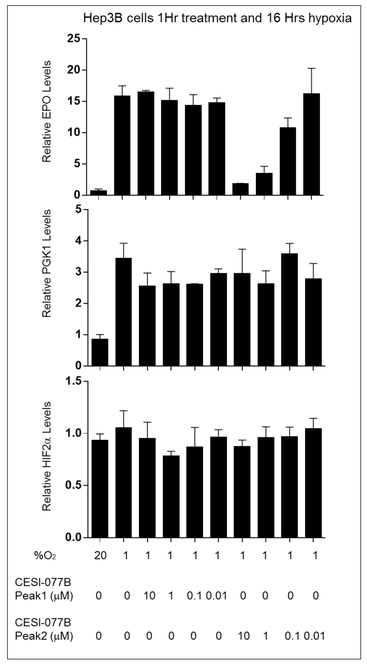


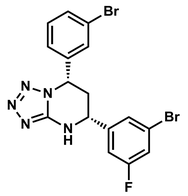

## 2014 AlphaScreen Data

| Compound ID | WT HIF2a | S304M control | 2014 ITC Kd |
|---|---|---|---|
| C131 PEAK 1 | 112 nM | > 30 µM | 63 nM |
| C131 PEAK 2 | > 30 µM | > 30 µM | >> 2 uM |
| C188 PEAK 1 | > 30 µM | > 30 µM | >> 2 uM |
| C188 PEAK 2 | 97 nM | 10.1 µM | 165 nM |
| C189 PEAK 1 | 108 nM | > 30 µM | 109 nM |
| C189 PEAK 2 | > 30 µM | > 30 µM | >> 2 uM |
| C197 PEAK 1 | 87 nM | 23.9 µM | 125 nM |
| C197 PEAK 2 | > 30 µM | > 30 µM | >> 2 uM |
| CESI-0778 PEAK 1 | > 30 µM | > 30 µM | >> 2 uM |
| CESI-0778 PEAK 2 | 43 nM | 14.5 µM | 23 nM |
| DS-01-145 PEAK 1 | 32 nM | 3.8 µM | **24 nM** *** |
| DS-01-145 PEAK 2 | 22.3 µM | > 30 µM | >> 2 uM |
| DS-01-57 PEAK 1 | > 30 µM | > 30 µM | >> 2 uM |
| DS-01-57 PEAK 2 | 195 nM | 15.9 µM | 294 nM |
| DS-01-65 PEAK 1 | 28.3 µM | > 30 µM | >> 2 uM |
| DS-01-65 PEAK 2 | 314 nM | 18.9 µM | 360 nM |
| DS-01-91 PEAK 1 | > 30 µM | > 30 µM | >> 2 uM |
| DS-01-91 PEAK 2 | 1.0 µM | > 30 µM | 1239 nM |
| DS-01-97 PEAK 1 | 248 nM | > 30 µM | 487 nM |
| DS-01-97 PEAK 2 | > 30 µM | > 30 µM | >> 2 uM |

*** saturates rather late in the titration

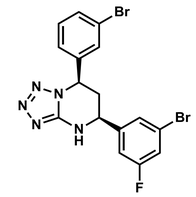
## Cellular Hypoxic Response *in vitro*



CESI-077B-Peak 1

*inactive enantiomer*



CESI-077B-Peak 2

*active enantiomer*

Diagram 1

## Conclusions

It was known that due to the nature of the binding site, only one enantiomer of these compounds is an active inhibitor of the HIF2α dimerization. As shown in *Diagram 1*, this family of compounds has a powerful inhibitory effect on the HIF2α transcription factor selectively by blocking EPO induction while having no effect on the HIF1α induction protein PGK1.

The affinity of different compounds within the family for the binding site of wildtype HIF2α were compared by both Alphascreen technology and isothermal calorimetry studies giving reasonably correlating results preliminarily. Furthermore, the mutant type HIF2α having a point mutation to fill in the site, shows drastically reduced drug binding activity, as expected.

## Future work

1. Developing an asymmetric synthetic scheme to obtain stereochemically pure product not requiring enantiomeric separation.

2. Developing a new scaffold of this family of compounds using the higher affinity compounds as a starting points.

3. Continuing biochemical studies to further plot the effects, including possible off-target effects, of this family of compounds on human cells.

4. Application of the compounds in *in vitro* cancer study projects for malignant cell lines that have aberrant activation in HIF2α.

### References

1. Yang C, Sun MG, Matro J, Huynh TT, Rahimpour S, Prchal JT, Lechan R, Lonser R, Pacak K, Zhuang Z (March 2013). *"Novel HIF2A mutations disrupt oxygen sensing, leading to polycythemia, paragangliomas, and somatostatinomas". Blood* **121** (13): 2563–6.
2. Tambar et al, *Development of Inhibitors of the PAS-B Domain of the HIF-2α Transcription Factor*. dx.doi.org/10.1021/jm301847z | J. Med. Chem. 2013, 56, 1739−1747
3. Tambar et al. *Allosteric inhibition of hypoxia inducible factor-2 with small molecules.* doi:10.1038/nchembio. N. Chem. Bio. 118.5. 271-276.