Understanding the Class of Thalidomide Analogs Through the Stabilization & Characterization of Their Interconverting Enantiomers

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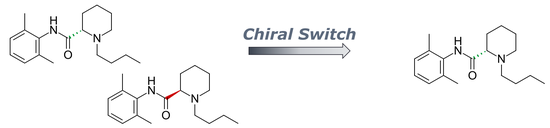
Background

Chirality and Chiral Switching

Chemical compounds and drugs can exist as non-superimposable mirror-images of each other (enantiomers). Several drugs have been successfully 'switched' from a mixture of two enantiomers to the single, preferred enantiomer based on its superior properties resulting in an improved therapeutic profile.

Example of a chiral switch: From a racemate to the single, preferred enantiomer



Bupivacaine (Marcaine®)
Racemate
(Mixture of (*R*)- & (*S*)-enantiomers)

Levobupivacaine (Chirocaine®)
(S)-enantiomer
less vasodilation, longer duration of action, longer motor block onset time

Deuterium-Enabled Chiral Switching (DECS)

Some marketed drugs and drug candidates are still mixtures of enantiomers because the chiral center is unstable and can interconvert *in vivo*. We have discovered the use of DECS to stabilize and differentiate the single enantiomers of many racemic compounds. Replacing hydrogen with deuterium results in chemical bonds that are up to 70x stronger, slowing the chemical interconversion of enantiomers. Several potential advantages include:

- Improved therapeutic index, novel mechanism of actions, new indications
- Speed to market & lower risk – anticipated accelerated regulatory path
- Compliance with FDA Guidance for 'The Development of New Stereoisomeric Drugs'
- New composition of matter patent protection
- Generally no impact on, or identical metabolism to parent racemic drug

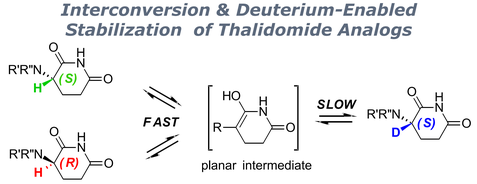
Thalidomide Analogs

Immunomodulatory drugs thalidomide, lenalidomide, and pomalidomide have demonstrated anti-tumorigenic and anti-inflammatory activity in human clinical trials and are approved for the treatment of hematological cancers. Their mechanism of action has been linked to their cereblon-binding activity. Recently, these compounds have also demonstrated strong synergistic effects for immuno-oncology applications when combined with immunomodulatory compounds (e.g. rituximab, pembrolizumab), and other cancer agents. Compounds CC-122 and CC-220 are examples of a new series of cereblon E3 ligase modulators that are in clinical development. CC-122 is being studied as a monotherapy and in combinations with rituximab, obinutuzumab, ibrutinib, nivolumab, sorafenib, the mTOR inhibitor CC-223, and the BTK inhibitor CC-292 for hematological cancers and solid tumors. CC-122 is more potent than lenalidomide and is being studied in clinical trials without dexamethasone.

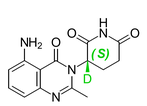
Deuterium Stabilization of Thalidomide Analogs

Some thalidomide analogs contain a rapidly interconverting chiral center. This has prevented a classical 'chiral switch' approach. We have used DECS to stabilize and characterize the enantiomers of lenalidomide [2] and several additional thalidomide analogs including CC-11006 [3], CC-122 [3], and CC-220 [4]. The stabilized (*S*)-enantiomer of CC-122 is DRX-164.

Interconversion & Deuterium-Enabled Stabilization of Thalidomide Analogs



DRX-164

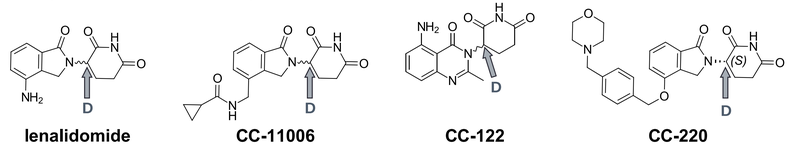


Methods

- Stability [2,3] was determined by incubation in phosphate-buffered saline (PBS), human or mouse plasma at 37°C and analysis by LC/MS-MS. Rate constants were obtained by numerical nonlinear regression of the LC/MS-MS data against differential kinetic equations (Excel solver).
- TNF-a inhibition [2,3] IC_{50}s were obtained by analysis of quantitative TNF-a data from 18h incubation of human peripheral blood mononuclear cells (PBMCs) stimulated with lipopolysaccharide (LPS) in the presence of increasing concentrations of test compounds (Hill equation with variable slope, GraphPad Prism 6).
- Cereblon-binding data were obtained in a fluorescent polarization probe displacement assay as described before [5] with the exception that the Cy5-thalidomide probe was replaced by an Atto565-lenalidomide probe.
- Pharmacokinetics (PK) studies were run in male rats (lenalidomide) or female SCID mice (CC-122) dosed orally with test compounds with quantitation of drug in plasma by chiral LC/MS-MS. PK parameters were derived using non-compartmental analysis.
- Xenograft studies [3] were performed in female SCID mice administered 10^7 RPMI-8226 or H929 tumor cells in Matrigel. When tumors reached 100-150 mm^2, animals were treated by daily oral gavage with test compounds. Body weights were measured periodically and tumor size was determined biweekly until study termination (42 and 24 days, respectively).

In Vitro Effects of Deuterium-Stabilized Enantiomers ...

- Improved *in vitro* stability



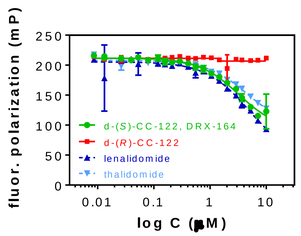
Compound	Decreased interconversion with D vs H			Degradation reduced with D vs H
	PBS	Mouse Plasma	Human Plasma	
d-lenalidomide [2]	3-5x	-	-	4-6x
d-CC-11006 [3]	-	-	~4x	3x
d-CC-122 [3]	-	1.5x	2-3x	-
d-CC-220	-	-	2-2.5x	-

- Anti-inflammatory effects are driven by the (*S*)-enantiomer

Compound	TNF-a inhibition, IC_{50} (nM)			
	Racemate	d-S	d-R	Fold difference (R/S)
d-lenalidomide [2]	30	8	-	-
d-CC-11006 [3]	-*	13.4	123	~10x
d-CC-122 [3]	-*	48.5	945	~20x

* TNF-a inhibition IC50 for racemic CC-11006 and CC-122 were determined, in separate assays, to be 50 nM [6] and 63 [7] nM, respectively.

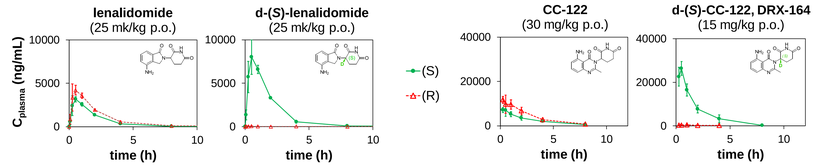
- Only the (*S*)-enantiomer of CC-122 binds to cereblon (displacement of a lenalidomide-based fluorescent probe) with similar potency to lenalidomide and thalidomide



Compound	Probe displacement, IC_{50} (μM)
d-(S)-CC-122, **DRX-164**	3.22
d-(R)-CC-122	none
lenalidomide	4.88
thalidomide	4.44

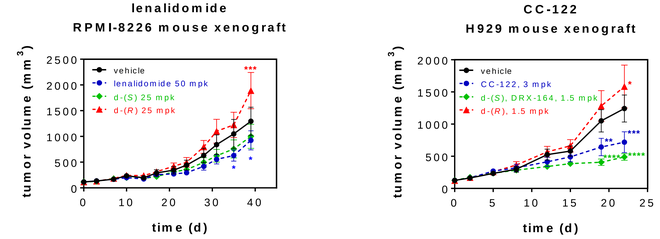
... Result in Significant *In Vivo* Benefits

- Almost exclusive exposure to preferred enantiomer



  - Racemate: stereoselective exposure for undesired (*R*)-enantiomer
  - d-(*S*): little to no D/H exchange → almost no exposure to undesired enantiomer
  - d-(*S*): no change in elimination half-life of deuterated vs protonated enantiomers

- Similar or improved efficacy in multiple myeloma models at equivalent dose



*Average tumor volume (± SEM) as function of time (2-way ANOVA with Dunnett's (lenalidomide) or Bonferroni's (CC-122) multiple comparison post-test against vehicle * P < 0.05, ** P < 0.01, *** P < 0.001, and **** P < 0.0001)*

  - Racemates show known, potent anti-tumorigenic activity (lenalidomide, CC-122)
  - (*S*)-enantiomer is anti-tumorigenic (lenalidomide, CC-122)
  - (*S*)-enantiomer is more potent than racemate at equivalent dose (CC-122)
  - (*R*)-enantiomer is potentially promoting tumor growth in these particular mouse models (lenalidomide, CC-122)

Conclusions

- **Deuterium stabilizes** enantiomers of thalidomide analogs against interconversion
  - Stabilization enables characterization of the properties of (*S*)- and (*R*)-enantiomers
- **Cereblon binding** due exclusively to (*S*)-enantiomer (CC-122)
- **Anti-inflammatory activity** consistently stronger for (*S*)-enantiomers
- **Pharmacokinetics** stereoselective after dosing deuterated (*S*)- or (*R*)-enantiomers
- **Anti-tumorigenic activity** in xenograft models of multiple myeloma
  - Efficacy due exclusively to (*S*)-enantiomers: similar or improved vs. racemate
  - Potential tumor-promoting effects due to (*R*)-enantiomers in these particular mouse models

(Note: The SCID mouse model does not account for the known immunomodulatory effects of drugs such as lenalidomide and does not, therefore, replicate all of the therapeutic effects observed in humans.)

Acknowledgments

We would like to acknowledge contributions to the synthesis and separation of the enantiomers from T.M. Judge, Ph.D. (Kalexsyn, Inc., Kalamazoo, MI) and L. Varady, Ph.D. (Rilas, Inc., Woburn, MA).

Notes & References

1. Deuteria Pharmaceuticals, Inc. was acquired and became a subsidiary of Celgene (2012 Form 10k).
2. Deuteria Pharmaceuticals, Inc. U.S. Patents #8,288,414, #8,669,276, and #9,023,868.
3. Jacques V. *et al* PNAS 2015, 112: E1471-E1479; DeuteRx, LLC U.S. Patent #9,540,340.
4. CC-220 is being developed as the (*S*)-enantiomer. It undergoes rapid interconversion to the (*R*)-enantiomer *in vitro* but minimal interconversion *in vivo*.
5. Fischer E.S. *et al* Nature 2014, 512: 49-53.
6. Data for racemic CC-11006 from pharmacology and toxicology review of lenalidomide NDA 021880.
7. Data for racemic CC-122 from Celgene U.S. Patent #8,906,932.