

# Synthesis and Stability of Oxetane Analog of Thalidomide and Lenalidomide

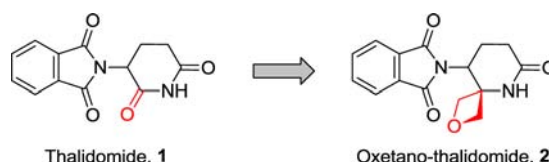
Johannes A. Burkhard,<sup>†</sup> Georg Wuitschik,<sup>‡</sup> Jean-Marc Plancher,<sup>‡</sup>  
Mark Rogers-Evans,<sup>\*,‡</sup> and Erick M. Carreira<sup>\*,†</sup>

Laboratorium für Organische Chemie, ETH Zürich, CH-8093 Zürich, Switzerland, and  
F. Hoffmann-La Roche AG, pRED, Discovery Chemistry, CH-4070 Basel, Switzerland

mark.rogers-evans@roche.com; carreira@org.chem.ethz.ch

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## ABSTRACT



Oxetanes are used in drug discovery to enable physicochemical and metabolic property enhancement for the structures to which they are grafted. An imide C=O to oxetane swap on thalidomide and lenalidomide templates provides analogs with similar physicochemical and *in vitro* properties of the parent drugs, with an important exception: oxetane analog 2 displays a clear differentiation with respect to human plasma stability. The prospect of limiting *in vivo* stability/metabolism, blocking *in vivo* racemization, and potentially altering teratogenicity is appealing.

Thalidomide, an antiemetic and sedative, was introduced in the late 1950s and early 1960s to devastating effect: more than 10 000 children in 46 countries were born with birth defects such as phocomelia, dysmelia, amelia, bone hypoplasticity, and other congenital defects affecting the ear, heart, or internal organs.<sup>1</sup> As a consequence of these tragic events, thalidomide's therapeutic use lay dormant. But, despite a ban, its pharmacology was revisited in a landmark study by Sheskin<sup>2</sup> in 1965 to effectively treat erythema nodosum leprosum (ENL). Later it was determined that thalidomide leads to a decrease in TNF- $\alpha$  levels by enhancing the degradation of its mRNA, and subsequently it was approved by the FDA in 1998 with strict prescription controls. In 1994, studies suggested that thalidomide-linked malformations were the result of the drug's interference with vasculogenesis and that a similar mechanism might prevent the growth of blood vessels in solid tumors. This led to the 2006 accelerated FDA approval of thalidomide in combination with dexamethasone for the treatment of multiple myeloma. It is under investigation for various types of cancer, as well as for inflammatory conditions,<sup>3</sup> although, until recently, the

molecular basis of its teratogenicity has been unclear.<sup>4</sup> Further studies<sup>5</sup> have revealed that thalidomide is a potent angiogenesis inhibitor *in vivo*, but no effect was observed on the proliferation of endothelial cells in culture, leading to the postulation that a *thalidomide metabolite* might be the active agent. This was later demonstrated by determining that the addition of human liver microsomes was essential to observe an antiangiogenic effect of thalidomide in *ex vivo* studies.<sup>6</sup> The two enantiomers of thalidomide undergo ready interconversion *in vivo*, and each elicit distinct effects with (+)-*R* as a sedative and (–)-*S* as a teratogen.

The metabolic modification of thalidomide and its susceptibility to racemization led us to consider the prospect of a carbonyl to oxetane switch to control both: altering the *in vivo* metabolic profile and rendering the compound configurationally stable.<sup>7</sup> As a first step to

(4) Ito, T. *Science* **2010**, 327, 1345.

(5) D'Amato, R. J.; Loughnan, M. S.; Flynn, E.; Folkman, J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, 91, 4082.

(6) (a) Bauer, K. S.; Dixon, S. C.; Figg, W. D. *Biochem. Pharmacol.* **1998**, 55, 1827. (b) Lepper, E. R.; Smith, N. F.; Cox, M. C.; Scripture, C. D.; Figg, W. D. *Curr. Drug Metab.* **2006**, 7, 677. (c) Kumar, N.; Sharma, U.; Singh, C.; Singh, B. *Curr. Top. Med. Chem.* **2012**, 12, 1436.

(7) Oxetanes have been shown to have increased metabolic stability relative to other cyclic ethers: Stepan, A. F.; Karki, K.; McDonald, W. S.; Dorff, P. H.; Dutra, J. K.; DiRico, K. J.; Won, A.; Subramanyam, C.; Efremov, I. V.; O'Donnell, C. J.; Nolan, C. E.; Becker, S. L.; Pustilnik, L. R.; Sneed, B.; Sun, H.; Lu, Y.; Robshaw, A. E.; Riddell, D.; O'Sullivan, T. J.; Sibley, E.; Capetta, S.; Atchison, K.; Hallgren, A. J.; Miller, E.; Wood, A.; Obach, R. S. *J. Med. Chem.* **2011**, 54, 7772.

<sup>†</sup> ETH Zürich.

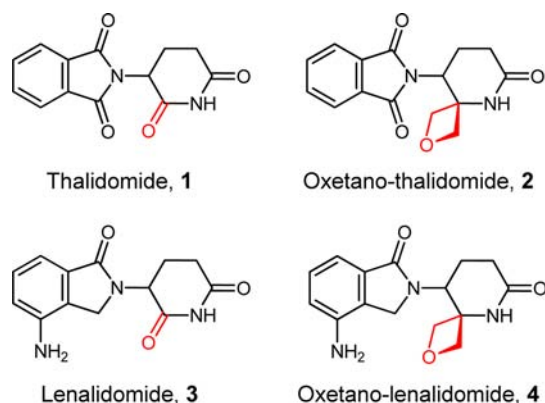
<sup>‡</sup> F. Hoffmann-La Roche AG.

(1) Matthews, S. J.; McCoy, C. *Clin. Ther.* **2003**, 25, 342.

(2) Sheskin, J. *Clin. Pharmacol. Ther.* **1965**, 6, 303.

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address these issues, this communication describes our preliminary investigation of both enantiomers of the oxetane analogues of thalidomide and its close congener lenalidomide (Figure 1), which was approved for the treatment of multiple myeloma in 2004 and under clinical investigation for a variety of other cancers, together with a comparison of their physicochemical and *in vitro* metabolic properties.



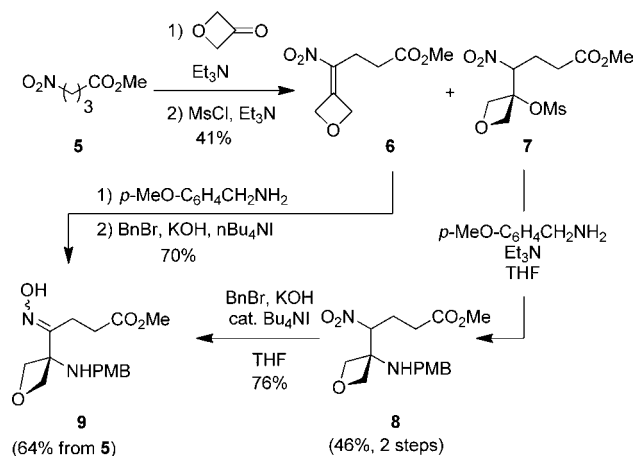
**Figure 1.** Thalidomide, lenalidomide, and oxetane analogues.

The synthesis of the oxetane analogue **2** commenced with a Henry addition of methyl  $\gamma$ -nitrobutanoate (**5**) and oxetan-3-one,<sup>9</sup> a reaction conducted neat in the presence of 0.2 equiv of  $\text{Et}_3\text{N}$  (Scheme 1). The 3-oxetanyl alcohol formed in situ was dissolved in  $\text{CH}_2\text{Cl}_2$  and treated at low temperature with  $\text{MsCl}$  and  $\text{Et}_3\text{N}$ . Slow warming of the reaction mixture and quenching upon reaching ambient temperature was necessary for optimal yield of **6**. When stirring is allowed to continue at room temperature, significant amounts of a rearranged isoxazole compound are otherwise formed, as previously described.<sup>10</sup> At this point chromatography on silica gel afforded two product fractions, containing nitroalkene **6** as well as a mixture of **6** and mesylate **7**. These fractions were independently processed en route toward **2**.

Treatment of a 1:4 mixture of nitroalkene **6** and mesylate **7** with  $\text{Et}_3\text{N}$  and 4-methoxybenzylamine in THF afforded 3-aminooxetane **8** in 46% yield, a compound which is also readily formed when **6** is separately treated with 4-methoxybenzylamine. All attempts at forming the  $\delta$ -lactam at this stage produced extensive decomposition of starting material. Consequently, we focused on the manipulation of the nitro group. Although reduction to the corresponding amine was possible (e.g., with  $\text{Zn}$ , aq.  $\text{HCl}$  in *i*-PrOH), the outcome of these reactions was unsatisfactory. Therefore, we decided to attempt partial reduction of the nitro

group. The mild conditions we have previously reported for the conversion of primary nitro groups to the corresponding oximes<sup>11</sup> ( $\text{BnBr}$ ,  $\text{KOH}$ , cat. TBAI) were successfully applied to the nitro group present in **8**, and the oximes **9** (as a 1:1.7 stereoisomeric mixture) were isolated in 76% yield and an overall yield of 64% from **5**.

#### Scheme 1. Pathways toward Oximes **9**



Subsequent heating of oximes **9** in xylenes led to the formation of lactam **10**, which was isolated as a single diastereomer in 84% yield (Scheme 2).<sup>12</sup> Reduction of the oxime with Raney-Ni under an atmosphere of  $\text{H}_2$  delivered amine **11** in excellent yield. Amine **11** was treated in the subsequent step with phthaloyl chloride/ $\text{Et}_3\text{N}$ , followed by DBU,<sup>13</sup> leading to clean formation of phthalimide **12** in 82% yield.<sup>14</sup> Finally, deprotection of the PMB-amide with  $\text{CAN}$  in  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  gave thalidomide analog **2** (54% yield).

We envisioned that amine **11** would also provide access to the oxetane analog (**4**) of lenalidomide (**3** in Figure 1). Consequently, **11** was treated with substituted benzyl bromide **13**<sup>15</sup> and  $\text{Et}_3\text{N}$  in hot DMF to afford the isoindolin-1-one **14** in 73% yield (Scheme 3).<sup>16</sup> Deprotection of the PMB group was effected with  $\text{CAN}$  in aqueous  $\text{CH}_3\text{CN}$  to give free amide **15** (43% yield). Reduction of

(11) (a) Czekelius, C.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2005**, *44*, 612. (b) Diethelm, S.; Carreira, E. M. *J. Am. Chem. Soc.* **2013**, *135*, 8500.

(12) We were unable to determine the stereochemistry of the oxime. It is tentatively assigned as the energetically favored (*E*)-isomer.

(13) (a) Myers, A. G.; Gin, D. Y.; Rogers, D. H. *J. Am. Chem. Soc.* **1993**, *115*, 2036. (b) Myers, A. G.; Gin, D. Y.; Rogers, D. H. *J. Am. Chem. Soc.* **1994**, *116*, 4697.

(14) It is worth noting that heating amine **11** with phthalic anhydride in toluene led to extremely sluggish product formation, presumably due to the sterically hindered nature of the amino group in **11**.

(15) Prepared from the corresponding toluene in a radical bromination: Röhrig, S.; Jeske, M.; Perzborn, E.; Gnoth, M. J.; Perzborn, E. (Bayer Healthcare AG). US2010010060, 2010.

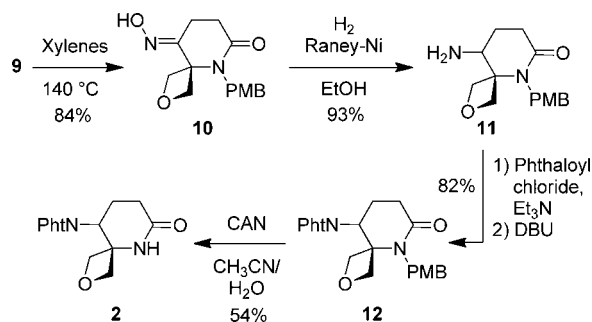
(16) This type of transformation is frequently used in the synthesis of lenalidomide and its analogues. Selected references: (a) Muller, G. W.; Chen, R.; Huang, S. Y.; Corral, L. G.; Wong, L. M.; Patterson, R. T.; Chen, Y. X.; Kaplan, G.; Stirling, D. I. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1625. (b) Muller, G. W.; Stirling, D. I.; Chen, R. S.-C. (Celgene Corp.). US 6335349, 2002. (c) Fujimoto, H.; Noguchi, T.; Kobayashi, H.; Miyachi, H.; Hashimoto, Y. *Chem. Pharm. Bull.* **2006**, *54*, 855.

(8) Prepared according to: Simoni, D.; Rondanin, R.; Morini, M.; Baruchello, R.; Invidiata, F. P. *Tetrahedron Lett.* **2000**, *41*, 1607.

(9) We are grateful to Dr. Thomas Fessard at SpiroChem AG, Zurich, Switzerland for providing oxetan-3-one as a gift.

(10) Burkhard, J. A.; Tchitchanov, B. H.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2011**, *50*, 5379.

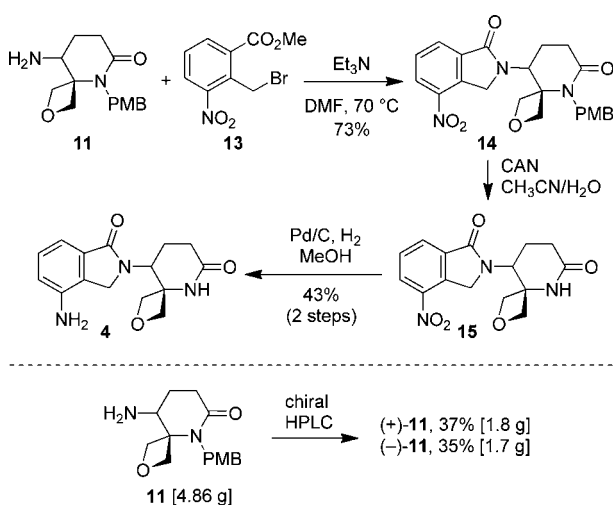
**Scheme 2. Synthesis of Oxetano-thalidomide, 2**



the nitro group was readily accomplished with Pd/C and H<sub>2</sub>, and oxetano-lenalidomide (**4**) was obtained in 99% yield.

The synthesis of the described oxetane analogs of both thalidomide and lenalidomide produced the targeted compounds in racemic form. However, because the complex pharmacology of thalidomide results from the differential effects associated with the separate enantiomers and their metabolic byproducts, we sought to gain access to enantiomerically enriched forms of the oxetane analogues. Unfortunately, separation of the target compounds **2** and **4** through the implementation of chiral HPLC was difficult because of issues of solubility. Accordingly, other intermediates were examined for resolution. Amine **11** was found to be readily separable by chiral HPLC, thereby giving gram quantities of (+)-**11** in 37% yield and (–)-**11** in 35% yield. Both enantiomers were subsequently taken forward to the corresponding thalidomide and lenalidomide analogs (see the Supporting Information for details).

**Scheme 3. Synthesis of Oxetano-lenalidomide, 4**



With the aim of profiling the oxetano-derivatives, physicochemical properties were evaluated and benchmarked against thalidomide and lenalidomide, all in their racemic forms (Table 1). Despite the introduction of the oxetane,

the N–H pK<sub>a</sub>'s were not significantly affected, and overall, all properties were not significantly altered, with the exception of reduced membrane permeation for **4**, measured via PAMPA.

**Table 1. Physicochemical Properties of 1–4**

	log D <sup>a</sup>	pK <sub>a</sub> <sup>b</sup>	sol. <sup>c</sup>	PAMPA <sup>d</sup>
<b>1</b>	0.24	10.7/-	29	3.5
<b>2</b>	0.0	>11/-	78	1.3
<b>3</b>	–0.64	10.4/2.6	100	0.47
<b>4</b>	–0.67	>11/2.6	100	0.06

<sup>a</sup> Logarithmic *n*-octanol/water distribution coefficient at pH 7.4.

<sup>b</sup> Ionization constants determined at 23 °C by spectrophotometry in water. <sup>c</sup> Intrinsic solubility measured at pH 6.5 by a Lyophilization Solubility Assay [μg/mL]. <sup>d</sup> Passive diffusion speed measured at pH 6.5 in a Parallel Artificial Membrane Permeation Assay [10<sup>–6</sup> cm/s]. For details, see the Supporting Information.

Similarly, the known low *in vitro* microsomal and hepatocyte intrinsic clearances of the parent compounds, thalidomide and lenalidomide, were predictably marginally affected by introduction of the oxetane and remained in a favorable range (Table 2) for the corresponding analogs. Additionally, 2C9, 2D6, and 3A4 cytochrome inhibition remained > 50 μM for oxetane derivatives **2** and **4**, thus maintaining the reduced risk for drug–drug interactions.

**Table 2. Intrinsic Clearance in Microsomes and Hepatocytes**

	microsomal clearance <sup>a</sup>			human hepatocyte clearance <sup>b</sup>
	human	mouse	rat	
<b>1</b>	6	4	9	3.7
<b>2</b>	5	5	6	2.3
(+)- <b>2</b>	2	6	6	2.6
(–)- <b>2</b>	3	3	7	2.5
<b>3</b>	1	2	0	1.5
<b>4</b>	3	3	1	0.17
(+)- <b>4</b>	0	0	0	0.23
(–)- <b>4</b>	0	0	-	0.40

<sup>a</sup> Intrinsic clearance rates [μL/min/mg] measured in human, mouse, and rat liver microsomes. <sup>b</sup> Intrinsic clearance rates [μL/min/10<sup>6</sup> cells] measured in human hepatocytes. For details, see the Supporting Information.

Screening of the derivatives in a human plasma stability assay, which is useful for interpreting follow-up *in vivo* efficacy, toxicity, and pharmacokinetic studies, proved intriguing (Table 3). In particular, thalidomide was unstable in comparison to its oxetane counterpart. It is therefore reasonable to expect that the latter's *in vivo* pharmacokinetic and toxicity profile will also be critically differentiated (studies pending).

Thalidomide and lenalidomide are therapeutically useful, but their patient distribution is severely limited due to

**Table 3.** Stability in Human Plasma<sup>a</sup>

	incubation time (h)			category
	0.25	1	5	
<b>1</b>	100	69	40	<b>unstable</b>
<b>2</b>	100	76	74	stable
(+)- <b>2</b>	100	74	72	stable
(-)- <b>2</b>	100	102	68	stable
<b>3</b>	100	93	63	stable
<b>4</b>	100	112	77	stable
(+)- <b>4</b>	100	124	68	stable
(-)- <b>4</b>	100	117	71	stable

<sup>a</sup>Stability in human plasma after 0.25, 1, and 5 h incubation time, expressed as a percentage of the initial concentration. The reported values represent the average of three runs ( $N = 3$ ). For details, see the Supporting Information.

their inherent teratogenic properties. This negative aspect might be ameliorated by controlling their *in vivo* stability/metabolism and/or their proclivity to racemization. The use of oxetanes as carbonyl surrogates<sup>17</sup> offers a novel ap-

(17) (a) Wuitschik, G.; Rogers-Evans, M.; Buckl, A.; Bernasconi, M.; Märki, M.; Godel, T.; Fischer, H.; Wagner, B.; Parrilla, I.; Schuler, F.; Schneider, J.; Alker, A.; Schweizer, W. B.; Müller, K.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2008**, *47*, 4512. (b) Wuitschik, G.; Carreira, E. M.; Wagner, B.; Fischer, H.; Parrilla, I.; Schuler, F.; Rogers-Evans, M.; Müller, K. *J. Med. Chem.* **2010**, *53*, 3227. For a review on the use of oxetanes in synthesis and drug discovery, see: (c) Burkhard, J. A.; Wuitschik, G.; Rogers-Evans, M.; Müller, K.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2010**, *49*, 9052.

proach to potentially control both of these factors. In summary, we have prepared the oxetane analogs of thalidomide and lenalidomide and effected their resolution. The comparative study reveals no significant differences in their physicochemical and *in vitro* metabolic profiles. However, compound **2** is strongly differentiated from thalidomide (**1**) in human plasma. The experiments underscore unexpected benefits of oxetanes in drug discovery that surface in this case for plasma stability. Efforts are ongoing in the design of experiments to determine the *in vivo* stability/metabolic profiles of the compounds synthesized as well as assaying their teratogenic potential.

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**Supporting Information Available.** Experimental procedures and characterization for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare the following competing financial interest: E.M. C. sits on the Board of SpiroChem AG, the company that provided oxetane-3-one as a gift (see ref 8).