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The synthesis and fluorescence profile of novel thalidomide analogues

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ABSTRACT

Herein we describe the synthesis of various simple *N*-alkyl thalidomide derivatives in order to determine their fluorescence excitation and emission profile. Following this, a series of more complex fragments were attached through a Huisgen 1,3-dipolar cycloaddition providing a more diverse fluorescence profile. A thalidomide azide derivative was also found to be particularly reactive in a copper-free click reaction with two known cyclooctynes.

1. Introduction

Thalidomide, [(*R*,*S*)-2-(2,6-dioxo-3-piperidinyl)-1*H*-isoindole-1,3(2*H*)-dione (**1**) (Fig. 1)] is perhaps one of the most known pharmaceuticals to chemists and biochemists alike. This drug was administered in the 1950's to pregnant woman both as a treatment for insomnia, and as an antiemetic barbiturate replacement. Tragically, this drug was released prior to the full understanding of the pharmacological profile of both enantiomers.[1] Recently, thalidomide (**1**) and the amino analogues lenalidomide (**2**) and pomalidomide (**3**) (IMiDs), have been used as a treatment for multiple myeloma, an as yet incurable form of bone marrow cancer.[2]

Thalidomide (**1**) and its analogues influence a wide range of cellular processes where initial investigations were primarily focused on the influence on thalidomide's influence on the pro-inflammatory cytokine, tumour necrosis factor (TNF). Specifically, this mode of action is thought to involve TNF expression and the inflammatory NFkB signaling pathway, specifically inhibiting the activity of an IkB kinase, IKK. More recently, there has been a specific focus on the elucidation of the teratogenic molecular modes of action of thalidomide, lenalidomide and pomalidomide (**1**–**3**), with findings having revealed the protein cereblon (CRBN), which is part of an E3 ubiquitin ligase, as one of the primary molecular targets of IMiDs.[3] Moreover, the crystal structures of this molecular target in

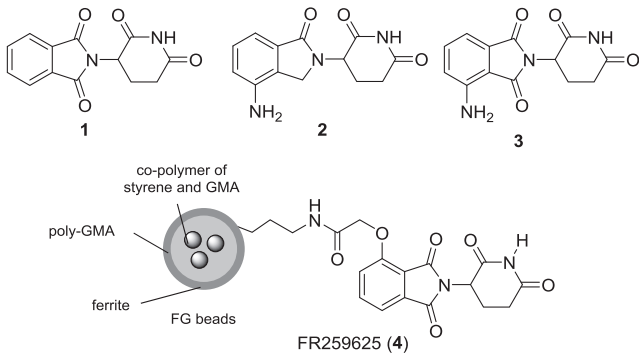

Fig. 1. Thalidomide (**1**), lenalidomide (**2**) and pomalidomide (**3**) and thalidomide derivative attached to FG beads (**4**).[7]

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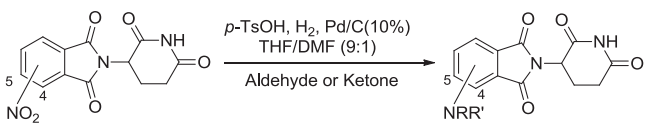
complex with either thalidomide or its analogues have been reported, disclosing the exact nature of the drug–protein binding interactions.[4,5] Furthermore, CRBN has been implicated in the immunomodulatory and antiproliferative activities of lenalidomide (**2**) and pomalidomide (**3**).[6] In the seminal study by Ito and coworkers, CRBN was isolated by using an ether linked thalidomide molecular probe attached to immobilized FG beads (FR259625, **4**).[7]

Our group has prepared a range of thalidomide analogues to inhibit the expression of TNF and related immunomodulatory activity.[8–10] We have followed up this study with investigations with a set of the more promising analogues in a liver cancer study.[11] Along with these medicinal chemistry studies, we have also been investigating the physical characteristics of thalidomide derivatives, and efficient synthetic methods for preparing such analogues.[12] Various analogues have been used to attach a biotin tether through a Huisgen 1,3-dipolar cycloaddition or click reaction. In this study, we were interested in the inherent fluorescence profile of thalidomide analogues with and without tailored fluorescent tags. With the development of such tools these compounds could be used to better understand thalidomide and its respective pharmacology.

2. Results and discussion

The preparation and fluorescence of simple phthalimide derivatives has been recently reported, including in vitro studies.[13] The fluorescence of phthalimides bearing secondary amines in the 4-position and the weak fluorescence of thalidomide have also been investigated.[13–16] We initially prepared a series of *N*-alkyl thalidomide analogues (**5**–**18**) containing substitution in the 4-position (similar to that of pomalidomide, **3**) or the 5-position. Subjecting 4- or 5-nitro thalidomide to a domino hydrogenation-reductive amination one-pot reaction, afforded several alkyl amine products (Table 1).[17] In general, the conversion to the alkyl amino thalidomide derivative was good to excellent, however, in the cases of reactions 7 and 9, Table 1, a mixture of mono and dialkylated products were obtained. In earlier attempts a small amount of the imine intermediate was also isolated as expected, given the required three step reduction, Schiff-base condensation and reduction reaction sequence.

Table 1
Synthesis and fluorescence features of *N*-alkyl thalidomide analogues



Reaction	Product	*N*-Subst	R	R′	Yield	λ_{ex} (nm)	λ_{em} (nm)
1	**5**	4	Et	H	98%	437	486
2	**6**	4	Pr	H	95%	445	487
3	**7**	4	Bu	H	79%	447	487
4	**8**	4	Pent	H	94%	446	487
5	**9**[17]	4	*i*Pr	H	30%	446	487
6	**10**	5	Et	H	65%	353	467
7	**11**	5	Pr	H	53%	349	469
	12	5	Pr	Pr	24%	441	489
8	**13**	5	Bu	H	33%	350	468
9	**14**	5	Pent	H	60%	351	467
	15	5	Pent	Pent	12%	442	493
10	**16**	5	*i*Pr	H	35%	344	467
11	**17**	5	Me	Me	90%	430	485
12	**18**	5	Et	Et	86%	436	487

The fluorescence excitation and emission maxima for compounds **5**–**18** are shown in Table 1. These can be clearly grouped by the compounds' substitution pattern. The C4 mono-alkyl amines (compounds **5**–**9**), except the *N*-ethyl tethered compound **5**, featured bimodal excitation spectra, with the global maximum centered around 446 nm and spectral feature peak around 378 nm (Fig. 2). The excitation spectrum of the 4-*N*-ethyl derivative **5** showed a broad excitation band with a maximum at 437 nm and a shoulder at 396 nm (Fig. 2). The emission spectra of the group of C4 *N*-alkyl compounds were almost identical with a single maximum, λ_{em} at 486 or 487 nm (Fig. 3).

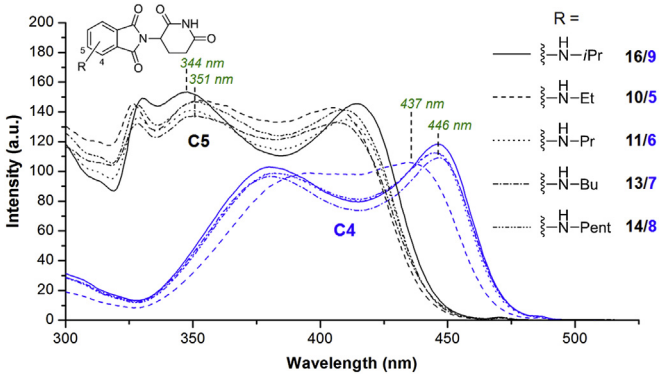


Fig. 2. Excitation spectra of 4- and 5-(alkylamino) substituted thalidomide analogues. All spectra were recorded at a concentration of 0.1 mg/mL in CH_2Cl_2. Global excitation maxima are indicated in green.

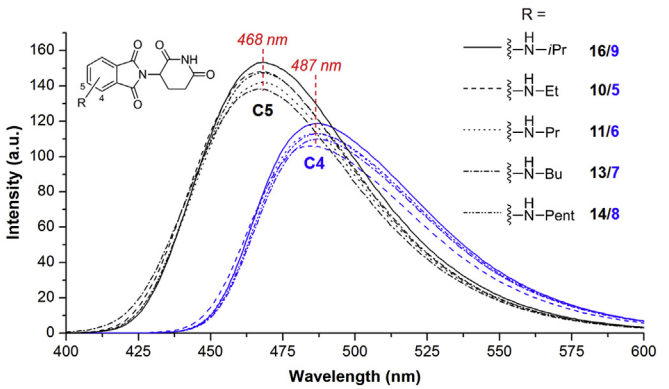


Fig. 3. Emission spectra of 4- and 5-(alkylamino) substituted thalidomide analogues. Each spectrum was obtained by using the maximum excitation wavelength as indicated in Fig. 2. All spectra were recorded at a concentration of 0.1 mg/mL in CH_2Cl_2. Average emission maxima are indicated in red.

The excitation spectra of the monoalkyl amines attached in the C5 position (compounds **11**, **13**, **14** and **16**) were all similar, and all featured nodes with peaks around 327, 350 and 410 nm (Fig. 2). In most cases, the maximum was around 350 nm. The emission spectra of the C5 compounds were again very similar with λ_{em} at slightly higher energy than the C4 analogues around 468 nm (Fig. 2). Interestingly, in many of these cases there was a large Stokes shift with this shift being up to 124 nm.

Like the 5-monoalkyl amine derivatives, the 5-*N*-dialkyl amine derivatives gave excitation spectra with several nodes, at around 300, 340, 360 and 430 nm (Fig. 4). In all cases, the excitation maximum near 430 nm was the most intense, with a slight shift to higher wavelengths with longer alkyl chain lengths. The emission spectra for these compounds were similar, however, with a trend of slightly increasing wavelength with increasing chain length.

Following the synthesis of the simple *N*-alkyl analogues, we decided to prepare thalidomide precursors which would allow attachment of known fluorescent agents. We conceived of linking the probe through alkylation of the phthalimide nitrogen, used

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